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SEP 22 1938

VOLUME 28 NUMBER 9

SEPTEMBER, 1938

Phytopathology

An International Journal

Official Organ of
The American Phytopathological Society

CONTENTS

The formation of colored zones by wood-destroying fungi in culture. HENRY HOPP 601

Seed transmission of Macrophomina phaseoli ..... C. F. ANDRUS 620

Annual growth rate of Cronartium ribicola cankers on branches of Pinus monticola in northern Idaho ..... T. S. BUCHANAN 634

Leaf blight of iris caused by Bacterium tardicrescens. LUCIA MCCULLOCH 642

Resistance and susceptibility to curly top in varieties of squash, Cucurbita maxima ..... B. F. DANA 649

The production of a new physiologic race of Sphacelotheca sorghi. SYED VAHEEDUDDIN 656

Phytopathological Notes 660

A race of Ustilago avenae capable of infecting Black Mesdag oats ..... EDWARD K. VAUGHAN

A seed-borne disease of sweet clover ..... FRED REUEL JONES

Report of the 1938 Annual Meeting of the Southern Division of The American Phytopathological Society 662

Report of the Twenty-second Annual Meeting of the Pacific Division of The American Phytopathological Society 668

Price { $6.00 per year, United States and dependencies,
$6.25 per year, Canada
$6.50 per year, elsewhere

PUBLISHED MONTHLY BY THE SOCIETY

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COR. NORTH QUEEN ST. AND MCGOVERN AVE., LANCASTER, PA.

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THE FORMATION OF COLORED ZONES BY WOOD-DESTROYING FUNGI IN CULTURE[1]

HENRY HOPP[2]

(Accepted for publication May 5, 1938)

A number of investigators (1, 3, 4, 5, 6, 7, 10, 11, 12, 13, 16, 20) have noted the presence of discolored zones[3] beneath the exposed surfaces of decayed wood that were subjected to drying. The discoloration in these cases was ascribed to the presence of masses of brown material in the wood elements. In most cases the observations were made on pieces of wood removed from decayed trees and allowed to dry in the laboratory. In a few cases (1, 4, 5, 10, 13), the studies were conducted with wood blocks that had been decayed by fungi growing in pure culture. Although the occurrence of discolored zones beneath the dry, exposed surface of decayed wood blocks has been observed frequently, no experimental work has been performed to determine the factors that dispose the mycelium to react in this manner. Various theories have been postulated, however, by observing the conditions that accompany the occurrence of the phenomenon. From such evidence certain workers (1, 2, 7, 12, 16, 21) have concluded that the zones consist of by-products resulting from the decomposition activities of the fungus; others (3, 4, 5, 6, 8, 13, 15, 20) have decided that the mycelium itself forms the brown masses that discolor the wood. The physiological causes of this type of zonation have been ascribed to the entrance of air into the wood (7, 11, 12, 16, 17), to desiccation of the mycelium (10), to unfavorable growing conditions for the fungus (3, 6, 20), to aging of the mycelium (13), and to deposition of toxic materials by the fungus (21).

There is considerable uncertainty as to the similarity of the discolored zones found in dried wood blocks and those associated with wood decay in

[1] This paper is a revision of a section of a thesis submitted in May, 1936, to the New York State College of Forestry, Syracuse, New York, as partial fulfillment of the requirements for the degree of Doctor of Philosophy. The investigation herein described was performed mostly in the Department of Forest Botany and Pathology under the supervision of Dr. Ray R. Hirt. Valuable suggestions were offered also by Dr. Henry F. A. Meier. A part of the work was done at the Botanisches Institut der Forstlichen Hochschule, Eberswalde, during 1932. To Dr. Johannes Liese, professor of the institute, thanks are due for active interest and advice.

[2] Associate Botanist, Section of Hill Culture Research, Soil Conservation Service, U. S. Department of Agriculture, Washington, D. C.

[3] The present confusion in terminology on this subject necessitates a delimitation of the terms used. Following is an attempt to select terms in conformity with present usage:

a) *Discolored zone:* An unnaturally colored region of the substratum the cause of which is associated with a pathological factor.

b) *Colored zone:* An organized group of pigmented hyphae located in the vegetative part of the hyaline mycelium. For this expression, Campbell (3) proposed and has subsequently used (4, 5) the term *pseudosclerotium.* It is considered advisable to exclude this term from the present discussion since the functional nature of the concept is inappropriate for describing the morphological aspect of the phenomenon.

c) *Zone line:* A discolored zone in the substratum that, when viewed macroscopically, appears as a narrow line. The use of the expressions *black line* and *black zone* for this term seems inadvisable since the color of the zones varies considerably under different conditions and with different fungi.

living trees. The possible relation between these phenomena emphasizes the necessity of obtaining a clearer understanding of the factors concerned in the formation of the discolored zones found in cultures. The determination of these factors was the object of the present investigation.

This study was performed by subjecting wood-destroying fungi in culture to various controlled environmental conditions. Since the discoloration referred to occurs in the form of restricted lines and narrow zones, it was apparent that those factors that vary quantitatively in different parts of the substratum probably induce the formation of zones. Light and heat conditions, which ordinarily are homogeneous within blocks decayed in culture, were, therefore, excluded *a priori* from consideration as the disposing factors in discolored-zone formation. Attention was given to other environmental factors, specifically to the effects of moisture and of the chemical composition of the atmosphere.

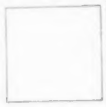
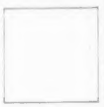
RELATIVE HUMIDITY PER CENT	SUBSTANCE USED FOR CONTROL	DISCOLORED ZONES AND PER CENT MOISTURE IN BLOCKS				
		FOMES APPLANATUS	FOMES FOMENTARIUS	FOMES FRAXINOPHILUS	POLYPORUS HISPIDUS	FOMES IGNIARIUS
100	DISTILLED H_2O	136	39	148	163	158
75	NA CL	84	 21	 61	90	136
53	$CA(NO_3)_2 \cdot 4H_2O$	50	13	19	89	118
35	$MgCl_2 \cdot 6H_2O$	9	8	7	8	15
0	ANHYDRONE	7	7	6	10	9

FIG. 1. Discolored zones in wood blocks of *Populus canadensis* var. *eugenie* subjected to decay by each of 5 species of fungi and aerated at various relative humidities. Each block shown is typical of a triplicate series. The diagrams are $\frac{1}{4}$ the actual size of the blocks.

EFFECT OF HUMIDITY SURROUNDING DECAYING BLOCKS ON ZONE FORMATION

Since previous observations indicated that discolored zones formed when decayed wood was subjected to desiccation, an attempt was made to determine the effect of the relative atmospheric humidity on the process. This was accomplished by subjecting decaying wood blocks to different relative humidities in 5 sterilized chambers. The air in the chambers was constantly renewed, and its humidity controlled by saturated aqueous solutions according to a method previously described and illustrated (9, p. 29 and Fig. 3). The relative humidities within the 5 chambers and the substances employed to secure these conditions are stated in figure 1.

Cubical wood blocks of *Populus canadensis* var. *eugenie* Schelle, which measured 5 cm. on a side, were used as a substratum for the fungi. The blocks were partially evacuated of air by soaking them alternately in boiling and cold water until they sank. Each block was then placed on a glass support, 15 mm. high, within a 500-ml. spoutless beaker, and water was added almost to the level of the bottom of the block. The top of the beaker was covered with a layer of absorbent cotton, which was held in place by the inverted half of a Petri dish. The culture blocks were then sterilized at 17 lbs. pressure for 30 minutes. After sterilization, small pieces of mycelium, removed from agar cultures, were placed on the blocks. The fungi on the wood blocks were then incubated in a saturated atmosphere, at 28° C., until the mycelium was established within the wood.

At the end of the incubation period (Table 1), some of the blocks were

TABLE 1.—*Pertinent data for experiment designed to test the relation between atmospheric humidity and the formation of discolored zones in wood blocks subjected to decay*

Fungus	Total number of blocks	Days of incubation in non-aerated vessels	Number of blocks examined at end of incubation period	Number of blocks with discolored zones at end of incubation period	Days the remaining 15 blocks were in the humidity chambers
F. applanatus	30	36	15	0	40
F. fomentarius	30	46	15	0	31
F. fraxinophilus	30	21	15	0	75
P. hispidus	30	37	15	0	39
F. igniarius	45	67	30	2	55

split open in order to determine whether discolored zones had formed. The remaining blocks were then subjected to controlled conditions in the humidity chambers, 3 blocks being placed in each chamber. After some days, as indicated in table 1, the blocks were removed from the humidity chambers and examined for the presence of discolored zones. The moisture content and specific gravity of a sample from each block also were determined. The following 5 species of fungi were used in the experiment: *Fomes applanatus* Pers., *F. fomentarius* (L.) Gill., *F. fraxinophilus* (Peck) Sacc., *Polyporus*

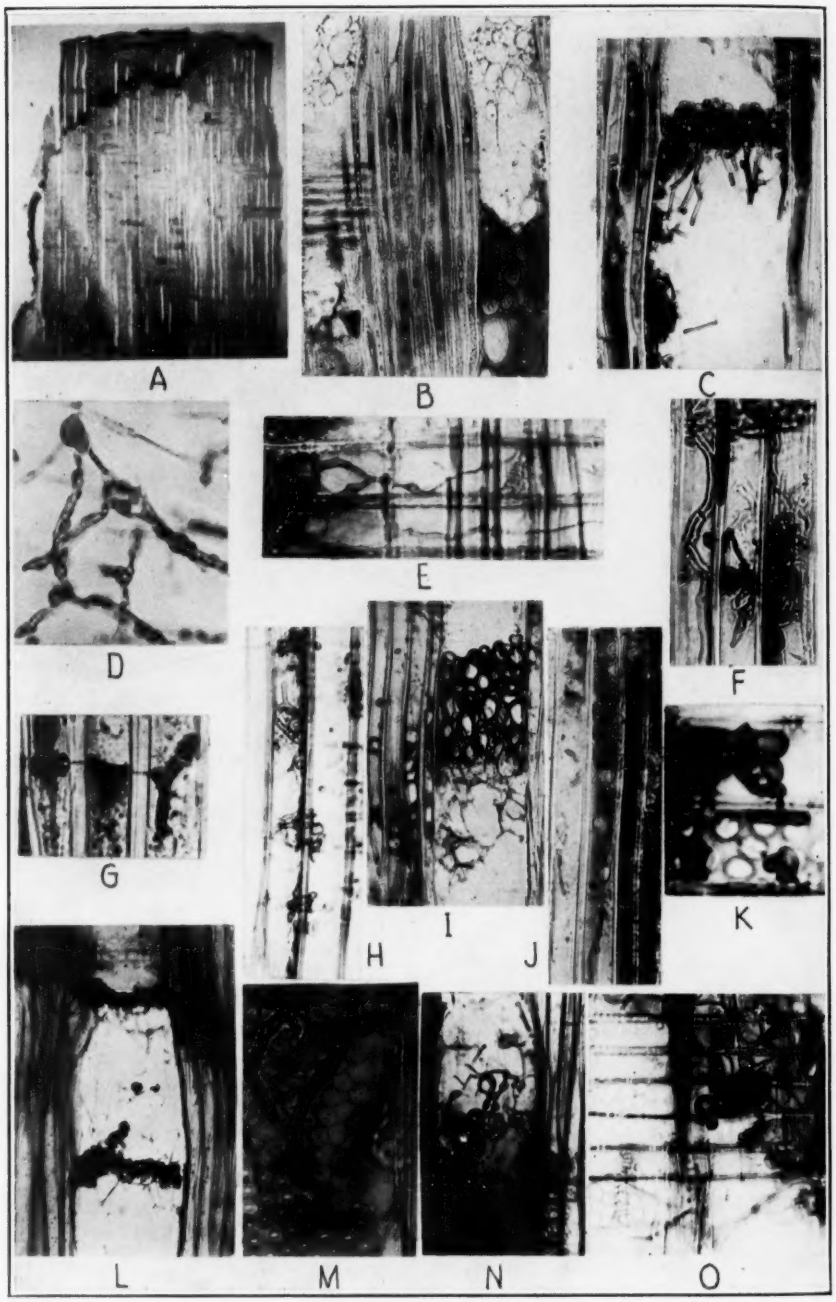


FIG. 2. Microscopic details of the colored zones formed in wood subjected to decay by various fungi in culture; A and B show wood of *Acer saccharum* Marsh., the remaining illustrations show wood of *Populus canadensis* var. *eugenie*. A, Zone line of *Fomes applanatus* beneath the exposed surface of a wood block, conterminous with the colored zone appearing in the mycelium on the outside of the block, ×6; B, Zone line of *F. applanatus*, consisting of swollen, colored hyphae, ×135; C, Zone line of *F. igniarius*, ×210;

hispidus (Bull.) Fries, and *F. igniarius* (L.) Gill. These species were selected because discolored zones frequently are associated with their presence in nature.

RESULTS

During the course of the experimental period, several changes were noted in the color of the mycelium on the surface of the blocks. Significant observations in this regard are described below for each species of fungus. The distribution of discolored zones within typical blocks decayed by each species of fungus is illustrated in figure 1.

Fomes applanatus. At the end of the incubation period in nonaerated vessels, most of the surface mycelium on the wood blocks was hyaline. In a few cases, liver-brown (19) patches had appeared.[4] When placed in the aerated humidity chambers, the blocks that were exposed to 0 per cent relative humidity immediately dried out on the surface and, therefore, did not change in external appearance. At the higher humidities, the external mycelium on these blocks became liver-brown. The color of the surface mycelium at the end of the experimental period is given in table 2. Microscopic examination showed that the colored surface mycelium consisted of swollen, tan-color hyphae.

TABLE 2.—*Color of the mycelium of Fomes applanatus growing on the surface of wood blocks aerated at various relative humidities*

Percentage relative humidity	Color of surface mycelium	
	Number of blocks with hyaline mycelium	Number of blocks with liver-brown mycelium
0	2	1
35	1	2
53	0	3
75	0	3
100	0	3

[4] Other blocks that had been subjected to decay by this fungus were incubated at 100 per cent relative humidity in sealed vessels for 77 days. At the end of this period, the mycelium on the external surfaces of the blocks had become only slightly pigmented.

D, Irregularly expanded zone-line hyphae of *F. igniarius* in a wood vessel, ×460; E, Hyphae of *F. igniarius* in a wood ray, showing various aspects of the mycelium during the development of a zone line, including unpigmented, filamentous hyphae at the right of the illustration, irregularly expanded hyphae in the center of the illustration, and swollen, colored hyphae at the left of the illustration, ×330; F, Longitudinal penetration of the wall of a wood fiber by a colored, zone-line hypha of *F. igniarius*, ×350; G, Transverse penetration of wood fibers by a colored, zone-line hypha of *F. igniarius*, showing constriction of the hypha within the walls, ×360; H, Transverse penetration of the walls of wood fibers by zone-line hyphae of *F. igniarius*, ×225; I, Zone line of *F. fomentarius*, consisting of colored, expanded hyphae, ×125; J, Details of zone-line hyphae of *F. fomentarius* in wood fibers, showing expanded hyphae and amorphous, brown fungous exeretions, ×185; K, Transverse penetration of the wall of a ray cell by the colored, expanded hyphae of *F. igniarius*, ×460; L, Zone-line mycelium of *Polyporus hispidus*, ×135; M, Zone-line hyphae of *F. fomentarius* penetrating the bordered pits of a wood vessel, ×250; N, Irregularly expanded zone-line hyphae of *F. fomentarius* in a wood vessel, ×150; O, Transverse penetration of the wall of a ray cell by the colored, expanded hyphae of *F. fomentarius*, ×207.

When the decaying blocks were split open at the termination of the experiment, zone-lines were found in several blocks. They were most prominent in those blocks that had been subjected to 75 per cent relative humidity. The lines were further below the exposed surface on the transverse faces, through which the external air could easily penetrate the wood, than on the longitudinal faces of the blocks. Where the lines extended to the external surface of the blocks, they formed the liver-brown patches previously referred to (Fig. 2, A). The hyphae constituting the colored zones appeared similar to those shown in figure 2, B.

Fomes fomentarius. During the period of incubation, profuse aerial mycelium grew over the surface of the blocks. This mat was removed when the blocks were placed in the humidity chambers. Subsequently, only a small amount of aerial mycelium formed. At 100 per cent relative humidity, a thin crust of brown mycelium formed over the surface of the blocks. A surface crust also occurred on portions of the blocks in 75 per cent relative humidity and on the top of 1 block in 53 per cent relative humidity.

Discolored zones were present inside 2 of the 3 blocks aerated at 100 per cent relative humidity. One of the blocks aerated at 53 per cent relative humidity also contained a light zone line. The zones, which were located just below the exposed surfaces of the blocks, consisted of swollen, colored hyphal masses (Fig. 2, I). The colored hyphae appeared similar to the mycelium constituting the surface crust.

Fomes fraxinophilus. Discolored zones occurred only in the blocks subjected to 100 per cent relative humidity. They were situated directly on, or just inside, the surface of the blocks. Microscopic observations showed that the zones consisted of masses of brown, swollen hyphae.

Polyporus hispidus. Discolored zones were present in some of the blocks in every chamber, but they were most prominent in the 3 blocks subjected to 75 per cent relative humidity. At 100 per cent relative humidity, discolored zones had formed in 2 of the 3 blocks. The 3 blocks exposed to 75 per cent relative humidity contained dark, prominent zones. At 53 per cent relative humidity, zones had formed in 2 of the 3 blocks. The blocks in 35 per cent and 0 per cent relative humidity contained a few, faintly-colored lines. The zones consisted of dark-brown, gnarled masses of hyphae (Fig. 2, L).

Fomes igniarius. Following several weeks of exposure to 100 per cent relative humidity in the fresh-air chambers, the surface mycelium became so profuse that the blocks were completely covered with a dense mass of brown hyphal filaments. The color of the mycelium was due to a brown pigment in the walls of the hyphae and to brown translucent droplets enclosed in the hyphal mass. The surface growth on the blocks placed in 75 per cent relative humidity was also profuse, but was only partly brown; most of the mycelium remained hyaline. Colored droplets exuded from the brown mycelium when it first formed, but after several weeks, the surface mycelium ceased growth and thereafter remained unchanged. The surface mycelium

on the blocks subjected to relative humidities of 53, 35, and 0 per cent dried out rapidly and remained hyaline. No new growth developed on these blocks after the first few days in the humidity chambers.

The occurrence of discolored zones in these blocks appeared inconsistent with the results obtained with the other species of fungi. The interiors of the blocks subjected to aeration at 100 and 75 per cent relative humidity were not discolored. Slight discoloration occurred inside only 1 of the 3 blocks exposed to 53 per cent relative humidity. Of the blocks placed in the chambers operated at 35 per cent relative humidity, 2 contained no zones, whereas the third block was prominently discolored. The wood just inside the exposed surface of the latter block was colored approximately cinnamon-buff (19). Farther into the block the wood was not discolored. A narrow zone line separated the discolored zone from the normally-colored portions. Microscopic observations showed that the colored line consisted of dark, thick-walled, gnarled hyphae (Fig. 2, C), present in the lumina of the wood elements. The discoloration of the wood between the zone line and the external surfaces of the block (Fig. 1) was due to the occasional presence of colored hyphae in the wood elements.

The interior of the 3 blocks subjected to 0 per cent relative humidity was discolored in a manner similar to that found in the block exposed to 35 per cent relative humidity. Several lighter colored, but otherwise typical, lines were found in the normally colored portion inside the discolored zones. The light color of these lines suggested that their maximum development had been inhibited, possibly by the rapid desiccation of the wood.

Although the humidity bore a definite relation to colored-zone formation by *Fomes igniarius*, the results were not in agreement with those obtained for the other fungi tested. On the basis of results thus far presented, this occurrence may be explained by either of 2 hypotheses. If specific percentages of relative humidity induced directly the process of colored-zone formation, the results of the experiment would indicate that *F. igniarius* reacted differently from the other fungi tested. On the other hand, if the effect of relative humidity were indirect, the results would indicate that the environmental complex that directly caused the formation of colored zones was distributed differently in the blocks decayed by *F. igniarius* than in the blocks decayed by the other fungi due to the physiological activity of the mycelium. The following 2 experiments were, therefore, undertaken in order to determine the effects of desiccation and aeration when applied directly to mycelium within the substratum. These tests indicated that the effect of relative humidity is indirect and that the second of the above hypotheses applied.

EFFECT OF HUMIDITY WITHIN DECAYING BLOCKS ON ZONE FORMATION

The effect of desiccation and aeration on the mycelium within blocks was determined by forcing a continuous stream of treated air under a slight pressure through decaying wood blocks by means of glass tubes that ex-

tended into the wood (Fig. 3). In this experiment, the mycelium within the wood was not subjected to various per cents of relative humidity. The object, rather, was to determine whether colored zones formed when hyphae in the wood elements were exposed (*a*) to aeration and desiccation, or (*b*) to aeration and moisture.

Cubical wood blocks of *Populus canadensis* var. *eugenie*, measuring 5 cm. on a side, were used. Two holes, 5 mm. in diameter and 25 mm. deep, were bored about 6 mm. apart on a radial surface of each block. Thick-walled, capillary, glass-tubing, 12 cm. long and 6 mm. in diameter, was forced into each hole. The blocks were then soaked alternately in hot and cold water

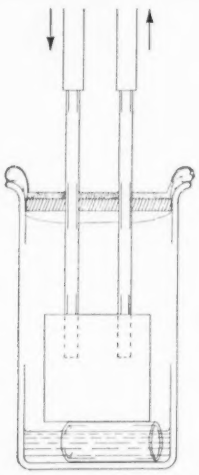


FIG. 3. Method used to aerate the hyphae growing within a wood block. × ⅓.

until they sank. Each block was placed on a glass support, 15 mm. high, within a 500-ml. spoutless beaker and water was added almost to the level of the bottom of the block. The top of the beaker was covered with a layer of absorbent cotton. A circular piece of heavy cardboard, through which 2 holes had been bored in order to accommodate the glass tubes, was placed over the cotton and was forced down on the flanged lip of the beaker. Ten blocks were thus prepared; 2 served as controls, 4 were aerated at 100 per cent relative humidity, and the other 4 were aerated at 0 per cent relative humidity. The inlet tubes on the 4 vessels to be treated with desiccated air were joined with glass T-tubes, so that the air stream would enter the blocks at an equal rate. The outlet tube from each vessel extended into a vial of sterilized water. The ends of the tubes that were to serve as inlet and outlet for the air stream were plugged with absorbent cotton. The 4 vessels comprising the 100 per cent relative humidity set-up were treated similarly. The rubber tubing on the glass rods of the remaining 2 vessels, used as controls, was plugged with cotton and sealed with metal clamps. The blocks were then autoclaved at 17 lbs. pressure for 30 minutes.

After sterilization, a small piece of mycelium from an agar culture was

placed on each block. Two fungi were used, *Fomes applanatus* and *F. igniarius*. Five blocks were decayed by each species of fungus according to the following plan: 1 control block, 2 blocks aerated at 100 per cent relative humidity, and 2 aerated at 0 per cent relative humidity. Following establishment of the fungi on the blocks, the culture vessels were hermetically sealed. The rubber tubes were closed with metal clamps and sufficient melted paraffin was poured over the cardboard cover of each vessel to form a thick layer. The cultures were then incubated at 28° C. for 35 days in order to permit establishment of the mycelium within the blocks. At the end of the incubation period, aeration of the culture blocks was begun.

The apparatus used to aerate the cultures at 0 per cent relative humidity consisted of a desiccating tower containing Anhydrone, the outlet from which was attached to a sterilized filter tube packed with absorbent cotton. The air, which was desiccated and sterilized in this apparatus, entered 1 set of 4 culture vessels, being diverted into each vessel through the T-tubes. The humidifying apparatus, a description of which has been given in a previous paper (9, Fig. 5), consisted of a glass tower containing moist linen cloth. The air stream, which was sterile and saturated with water as it came from the apparatus, passed into the other set of 4 culture vessels.

RESULTS

After 42 days of aeration, the blocks were removed from the culture vessels and were split open. The presence of colored zones was noted in the case of each fungus (Fig. 4).

Fomes applanatus. Inside the control block, which had been subjected

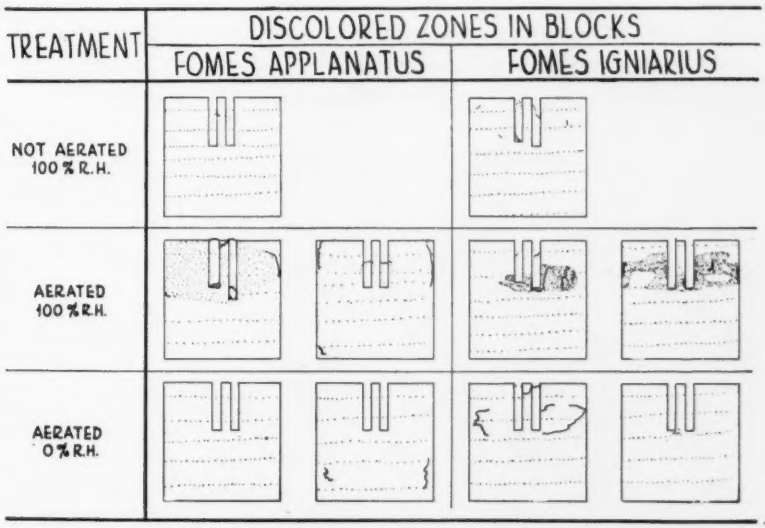


FIG. 4. Discolored zones inside decayed wood blocks of *Populus canadensis* var. *eugenie*, the interiors of which were aerated at 100 per cent and 0 per cent relative humidity. The dotted lines represent the longitudinal grain of the wood. The diagrams are ½ the actual size of the blocks.

to 100 per cent relative humidity but had not been aerated, 2 faint colored zones had formed. These were situated between the glass rods and the interior surface of the holes. In the 2 blocks aerated at 100 per cent relative humidity, prominent colored zones had formed. They were situated at the ends of the glass tubes in the wood, and in the portions of the wood where air from the holes could readily diffuse. The zones consisted of swollen, brown hyphae, similar to those shown in figure 2, B. In neither of the blocks aerated at 0 per cent relative humidity were any zones present in the vicinity of the holes. Zones were found, however, in the lower portion of 1 block. Their position in relation to the grain of the wood indicated that the dry air from the holes could not have diffused directly into the region where the lines occurred. In both blocks, the lower portions of the holes, which were not occupied by the glass tubes, contained a dense mass of hyaline mycelium.

Fomes igniarius. Colored-zone formation inside the control block was confined to a few, rather inconspicuous lines in the portion of the wood through which air from the holes could readily diffuse. In the blocks aerated at 100 per cent relative humidity, the wood around the tubes was markedly discolored. Several zone-lines were present in this region. The location of the discolored portion in relation to the grain of the wood indicated that the colored zones occurred in places directly exposed to the moist air stream. The discoloration was attributable to brown, gnarled masses of hyphae in the intracellular spaces of the wood. These hyphae were similar in appearance to those shown in figure 2, C. Only 1 of the blocks subjected to aeration at 0 per cent relative humidity had been dried out. In this piece, a few faint zones had formed. That the other block had not been completely dried out was evidenced externally by drops of condensed water, present on the inner walls of the culture vessel. This condition was caused by plugging of the glass tubes with a dense growth of mycelium. Although the drying effect on the outside of the block was slight, passage of dry air into the wood desiccated the region immediately around the glass tubes. This portion of the block was very dry and brittle, whereas the rest was wet. A prominent zone line demarked the boundary between the wet and dry portions of the block.

EFFECT OF HUMIDITY WITHIN DECAYING WOOD FLOUR ON ZONE FORMATION

The method of experimentation just described was applied in studying the effect of moisture and aeration on fungi growing in wood flour. The object in using wood flour was to permit observation of the mycelium during the process of colored-zone formation. This was accomplished by the procedure described below.

Round-neck bottles of 350-ml. capacity were used as culture vessels. Into each was placed about 200 ml. of wood flour, made from ground, air-dried wood of *Populus canadensis* var. *eugenie*. The material used was that that passed a 60-mesh standard sieve, but was retained by an 80-mesh sieve. Sufficient water was added to the culture vessels to saturate the wood flour. The vessels were stoppered with corks through which 3 holes had been bored. A

plug of absorbent cotton was placed in one of the holes. Glass tubes were extended through the remaining holes into the wood flour. The end of each tube was bent to a right angle, so that the opening of the tube just touched the inside wall of the bottle and was, therefore, visible externally. It was thus possible to observe the place where the conditioned air came in immediate contact with the wood flour.

Six culture vessels were thus prepared and were sterilized in an autoclave at 17 lbs. pressure for 30 minutes. Mycelium of *Fomes applanatus* was established on the wood flour in 3 of the bottles, and, in the 3 remaining bottles, *F. igniarius* was introduced. The mycelium was placed on the wood flour by momentarily removing the cotton plug from the hole that had been bored in the cork of each bottle. The corks were then sealed with paraffin in order that the only external supply of air to the mycelium was that entering the wood flour through the glass tubes. The 6 cultures were divided into 3 pairs, each of which consisted of 1 culture of *F. igniarius* and 1 of *F. applanatus*. The cultures were incubated for 42 days, during which time the ends of the glass tubes were sealed. At the end of this period, 2 pairs of cultures were subjected to aeration by forcing air into the wood flour through the glass tubes. Humidifying and desiccating apparatus similar to that used in the preceding experiment were employed. The remaining 2 bottles, which served as controls, remained sealed throughout the duration of the experiment.

RESULTS

After the air had been blown through the wood flour for 37 days, the experiment was terminated. Observations were made during this period in regard to the formation of colored zones by each of the fungi (Fig. 5).

Fomes applanatus. During the period of observation, no colored zones formed in the control culture.

After 19 days, the culture that was aerated at 100 per cent relative

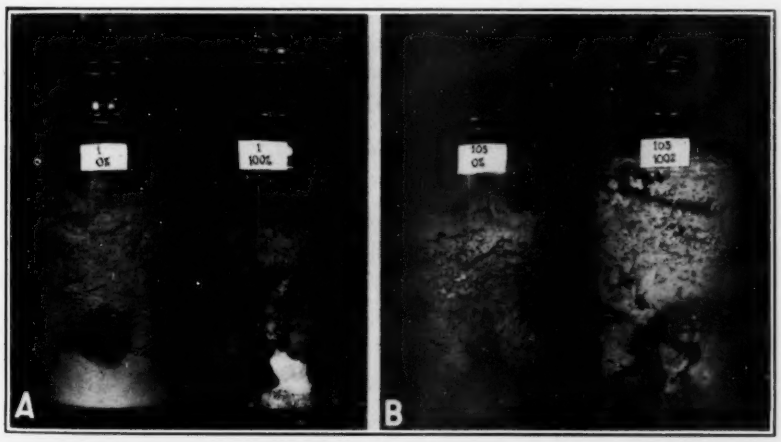


FIG. 5. Colored zones in wood-flour cultures which were aerated at 100 per cent and 0 per cent relative humidity. A, Cultures of *Fomes applanatus*. B, Cultures of *F. igniarius*. Control cultures (not shown in the illustration) did not discolor. $\times \frac{1}{3}$.

humidity contained a prolific growth of hyaline mycelium around the ends of the glass tubes. Some of these hyphae had become colored. The zones thus formed appeared on the inner wall of the bottle as dark lines. They consisted of brown, swollen, thick-walled hyphae of the type already described.

The wood flour in the culture aerated at 0 per cent relative humidity dried out without the formation of any zones. The wood surrounding the ends of the glass tubes became slightly discolored, but the typical colored zones, such as those present in the culture aerated at 100 per cent relative humidity, were not formed.

Fomes igniarius. No colored zones developed in the control culture during the period of the experiment.

In the culture aerated at 100 per cent relative humidity, prominent colored zones formed in the aerated region around the ends of the glass tubes. The zones appeared as irregularly distributed, dark lines consisting of masses of coarse, brown, thick-walled hyphae.

The wood flour in the culture aerated at 0 per cent relative humidity dried out rapidly. No colored zones formed.

EFFECT OF OXYGEN CONCENTRATION ON ZONE FORMATION

The previous experiments indicated that aeration of the mycelium was a controlling factor in colored-zone formation. An attempt was made, therefore, to determine whether the coloration was a response to the physical effect of air renewal or to the chemical action of the atmospheric gases.

The experiment was conducted by exposing mycelium to a reduced concentration of oxygen in an atmosphere saturated with moisture. The fungi were grown on wood flour in round-neck bottles, in a manner similar to that described in the previous experiment. Six bottles, connected together in pairs with T-tubes, were used. The wood flour in 1 bottle of each pair was decayed by *Fomes applanatus,* that in the other bottle by *F. igniarius.* The bottles were kept in an incubator at 28° C. for approximately 35 days. At the end of this time, the mycelium had become established throughout the wood flour in each bottle.

Following the period of incubation, saturated air containing oxygen of various concentrations was forced through the wood-flour cultures. One pair of cultures was exposed to air containing 20 per cent oxygen, 0 per cent carbon dioxide, and 80 per cent nitrogen. A second pair was treated with air containing 12 per cent oxygen, 8 per cent carbon dioxide, and 80 per cent nitrogen. The third pair was treated with oxygen-free air containing 2 per cent carbon dioxide and 98 per cent nitrogen. The cultures were observed for formation of colored zones.

RESULTS

Fomes applanatus

Twenty per cent oxygen. The culture was examined after 32 days of aeration. In this culture colored zones were present in the wood flour at the

ends of the glass tubes. The zones consisted of colored hyphae similar in appearance to those previously described for this fungus.

Twelve per cent oxygen. Colored zones formed also in this culture, but they were of a lighter color and were not so extensive as those that occurred in the culture exposed to air containing 20 per cent oxygen.

No oxygen. Air was blown through the wood flour for 38 days, but neither colored zones nor masses of hyaline mycelium developed. The appearance of such masses preceded the formation of colored zones in the other cultures.

Fomes igniarius

Twenty per cent oxygen. Prominent zones, consisting of colored hyphae similar to those previously described for this fungus, were present in the wood flour at the end of the 32-day period of aeration.

Twelve per cent oxygen. Several small zones formed in the wood flour. They were limited to the region in the immediate vicinity of the ends of the tubes.

No oxygen. No colored zones were noted in the wood flour after 38 days of aeration. The failure of this culture to form colored zones was not due to death of the mycelium, however, since subsequent aeration of the culture with air containing 12 per cent oxygen resulted in the formation of colored zones.

MORPHOLOGICAL NATURE OF COLORED ZONES

The typical morphological characteristics of the colored zones have been mentioned already. In the case of the 5 species of fungi studied, the zones consisted of swollen, gnarled hyphae showing various tones of brown. In some cases the hyphae were imbedded in a brown matrix. It was important to note that the microscopic appearance of the zones was characteristic for each species of fungus. The observation indicates the possibility of employing this character for the identification of vegetative mycelium growing in wood.

Formation of colored zones by *Fomes applanatus* began with expansion of the hyaline hyphae to a diameter of 20 to 30 μ. The swollen hyphae then became colored through the formation of a brown pigment. Colored material also was exuded into the lumina of the wood cells, the walls of which, however, did not become stained (Fig. 2, B).

Colored zones associated with *Fomes igniarius* formed in a somewhat similar manner. The zones began apparently with an increase in diameter and with pigmentation of the hyaline hyphae, portions of which then became enlarged to an average diameter of approximately 10 μ (Fig. 2, C, D, and E). The irregular expansions on the hyphae presented a gnarled appearance. At several of the places examined, the stout hyphae were colored but were not irregularly expanded. The pigment usually stained the cell walls of the wood and sometimes filled the lumina of the wood cells. Several peculiarities were noted in the form and location of the colored hyphae. In

places, colored hyphae had penetrated the pits of vessels, fibers, and ray cells (Fig. 2, H and K). Penetration of fibers by means of bore holes also was noted. According to Hubert (10, pp. 89 and 392), the bore holes produced by *F. igniarius* are very much larger than the hyphae. In the present study, this condition was usual throughout most of the decayed portion. The colored hyphae, however, usually were constricted where they had passed through the cell walls (Fig. 2, G). This observation indicates that in *F. igniarius* the type of cell-wall penetration is not constant. Another peculiarity was the longitudinal penetration of the middle lamella (Fig. 2, F), noted in both ray cells and fibers. Hubert (10) mentions only transverse penetration of the cell wall.

The hyphae constituting the colored zones of *Fomes fomentarius* (Fig. 2, I) developed from the hyaline hyphae apparently in a similar manner, that is, through swelling and pigmentation (Fig. 2, J and N). The swollen hyphae were about 20 μ in diameter, somewhat less than those of *F. applanatus*. Penetration of cell walls and pits by the zone-line hyphae was also observed (Fig. 2, M and O).

Formation of colored zones by other species was not studied in detail. Superficial examination of prepared slides indicated, however, that the zones of all the species studied had the same mode of formation. The zones of *Polyporus hispidus* (Fig. 2, L) and of *F. fraxinophilus* appeared somewhat similar to those of *F. igniarius*. The irregularly swollen hyphae that comprised the zones formed by these 3 fungi were approximately 10 μ in diameter, considerably less than that of the expanded hyphae of *F. applanatus* and *F. fomentarius*. The color of the zones differed with various fungi, being darkest in *F. igniarius* and *P. hispidus*, and lightest in *F. fraxinophilus*. The walls of the wood cells were discolored by *F. igniarius* and *P. hispidus*, but not by the other species studied.

DISCUSSION AND CONCLUSIONS

The results of these experiments showed for the 5 species of fungi tested that the occurrence of zones and lines of discoloration associated with wood decay was attributable to the formation of colored zones by the mycelium. The position of the colored zones, beneath and parallel to the exposed surfaces of the wood, indicated that the process of their formation was related to the external atmospheric conditions. Of these, aeration was apparently important, since coloration of the mycelium appeared in the blocks exposed within the aerated chambers, but did not generally form while the blocks were incubated in nonaerated vessels. Colored zones formed only when oxygen was contained in the air to which the mycelium was exposed. This indicated that coloration was a response to the chemical action of the atmospheric gases, not to the physical effect of gaseous renewal. The oxygen content of the atmosphere affected the prominence and probably the rate of colored-zone formation.

Likewise, the results definitely indicated that moisture relationships were

of significance in disposing the mycelium to form colored zones. The profuse growth and coloration of the surface mycelium on blocks aerated at 100 per cent relative humidity indicated that the presence of a saturated atmosphere was conducive to colored-zone formation. Since the zones within the blocks were confluent with and microscopically similar in appearance to the zones in the surface mycelium, it was to be expected that a high relative humidity would have the same disposing effect in their formation. The experimental results indicated this was apparently true for the fungi tested.

The effect of the external atmospheric humidity on the formation of colored zones within the blocks was apparently indirect in so far as it influenced evaporation of water and the consequent entrance of air into the wood elements. The proportion of air and water within the various blocks was approximated by moisture-content tests. The calculations indicated that colored zones did not occur in blocks that contained an extremely high concentration of water. This probably was because of the exclusion of the external air. The relation between the degree of saturation and the occurrence of colored zones was indicated particularly in the case of the blocks decayed by *Polyporus hispidus* at 100 per cent relative humidity. Two of the 3 blocks contained colored zones. These blocks had moisture contents of 163 and 167 per cent of the oven-dry weight. About 2/3 of the cellular space of the wood, therefore, was filled with water.[5] The third block, within which colored zones did not form, had a moisture content of 216 per cent of the oven-dry weight. Approximately 95 per cent of the cellular space contained water. It seemed probable that the concentration of water excluded air from the cellular spaces.

At humidities less than saturation, drying of the wood permitted external air to enter the cellular spaces and to come in contact with the mycelium. When the rate of desiccation was slow, as in the chamber operated at 75 per cent relative humidity, the mycelium in the cells of the wood was exposed to the external air and also was well supplied with water. It was under these conditions that the most pronounced formation of colored zones occurred. At the lower humidities, rapid drying permitted the entrance of air into the block, but free water was not long enough available to permit extensive zone formation. The necessity of interpreting the results in terms of the environment immediately surrounding the mycelium was noticed, particularly, in the case of the blocks decayed by *Fomes igniarius* in aerated chambers at various relative humidities. In this experiment, discoloration occurred only in blocks exposed to 35 and 0 per cents relative humidity. Since the

[5] The percentage of cellular space of the wood elements filled with water (W_f) was calculated by means of the following formula:

$$W_f = \frac{d \times sp.gr.(W_f - W_{f.s.p.})}{d - sp.gr.}$$

in which d is the density of the cell-wall substance; $sp.gr.$ is the specific gravity of the sample (oven-dry weight/wet volume); W_f is the moisture content of the fresh sample, expressed as percentage of the oven-dry weight; $W_{f.s.p.}$ is the moisture content of the sample at fiber-saturation point, expressed as percentage of the oven-dry weight.

other tests had shown that the formation of zones by *F. igniarius* actually was inhibited by desiccation of the mycelium, it seemed apparent that at the time the zones formed in the wood blocks the mycelium was not dry. Moisture-content determinations on blocks exposed to relative humidities above 35 per cent indicated that the relatively great concentration of water in these pieces prevented aeration sufficient for colored-zone formation. In the blocks exposed to 35 and 0 per cents relative humidity, the rate of evaporation permitted entrance of air into the wood, but was probably too slow to cause rapid desiccation of the mycelium.[6]

Consideration of the results here progressively described led to the conclusion that colored zones formed only when mycelium was exposed concurrently to large quantities of air and water. There was, however, a certain degree of incompatibility in these conditions, since wood containing a large amount of free water was almost devoid of air. On the other hand, if the wood contained a large amount of air, it held only a small amount of intracellular water. Therefore, situations where mycelium was exposed simultaneously to large amounts of both air and water were very specific. For this reason, coloration was generally restricted in location to definite areas and appeared as narrow zone lines.

This conclusion tends to explain some of the apparently contradictory results obtained by other workers. Harder (6), working with *Xylaria*, noted that zones occurred on the surface of decayed blocks of wood exposed to moist air. He also noted that colored zones formed beneath the exposed surfaces, that is, in the outer cell layers of dried wood blocks that had been placed under water. Harder inferred that these 2 sets of conditions were so completely opposed that the direct disposing factor could not have been exposure of the mycelium either to air in the one case or to water in the other. He decided, therefore, that the formation of colored zones is a type of vitalistic phenomenon, which he termed *Dauerzustand*. When interpreted according to the results obtained in the present investigation, his observations indicate that the zones formed as a response of the mycelium to definite environmental influences. Harder noted that the formation of surface zones on wood exposed to air occurred only when the blocks were not dried rapidly. The zones formed under conditions that indicated the aerial mycelium was moist and well supplied with air. That surface mycelium colors under these conditions also was observed in the present investigation, as shown in the case of *Fomes applanatus* growing on blocks exposed to a high relative humidity (Table 2). The occurrence of zones inside dry pieces of

[6] Other, unpublished data indicate that under conditions comparable with those of the present experiment, *Fomes igniarius* liberates a considerable quantity of free water. This result may account for the relatively high concentration of water in the blocks aerated at 53 per cent and 75 per cent relative humidity, and offers an explanation for the possibility that aeration at a low relative humidity desiccated the mycelium inside the wood at a slow rate.

The physiological characteristics of the mycelia presumably account for the significant differences in moisture content of the blocks decayed by the different species. The water metabolism of the fungi, through its effect on the moisture content of the substratum, was, therefore, an indirect factor of importance in colored-zone formation.

wood that had been placed in water can be explained in a similar manner. The mycelium that formed the zones was exposed to air and water, only the relative position of these 2 agents was reversed from that existing in the pieces exposed to moist air. The supply of water to the mycelium was on the outside of the block and the air was contained within the wood elements. This situation was similar to that described in the present investigation in which the interior of moist blocks was aerated with saturated air.

Hubert (10) observed the occurrence of zones beneath the exposed transverse surfaces of large pieces of decaying wood placed in the warm atmosphere of the laboratory. He also noted the presence of zones within dried blocks decayed by various fungi in culture tubes. This worker attributed the formation of the zones to desiccation of the mycelium. As previously noted, however, it is important to distinguish between external drying of the blocks and desiccation of the hyphae within the blocks, when analyzing the effect of an indirect agent. The results of the present study indicate that when desiccation of the mycelium occurs, the formation of colored zones is inhibited. The effect of external drying is to cause evaporation of water from the wood, as a result of which the mycelium is aerated. Under these conditions, desiccation of the hyphae inside the wood may be only slight. A similar interpretation may explain the common observation, mentioned by Hubert, that discolored zones form "in the upper portion of rotted stumps in the region where excessive drying occurs, roughly parallel to the surfaces from which evaporation takes place."

Prillieux (16) noted the occurrence of a related phenomenon on the exposed transverse surfaces of logs decayed by certain fungi. The exposed end of a log decayed by *Polyporus hispidus* was protected from drying by placing the section upright on a piece of glass. After several days, brown mycelium grew from the region of advanced decay and formed a mat between the wood and the glass. Brown droplets of a liquid were exuded from the mycelium. The writer has observed a similar phenomenon when pieces of aspen and beech wood, decayed in association with the presence of *Fomes igniarius*, were placed on the laboratory floor. Brown mycelium grew over the protected end of the log, between the exposed surface and the floor. The brown, coarse hyphae that constituted the superficial mycelium extended a short distance into the decayed interior of the section. The hyphae appeared similar to those that constituted the colored zones formed by this fungus in culture. Brown mycelium did not grow from the other transverse surface, which was directly exposed to the dry external air, but typical zones were found in the wood beneath this exposed portion. Microscopic examination indicated that the formation of a mat of colored hyphae on the exposed surface of a decayed block is a process similar to the formation of zones within wood tissue exposed to dry air. According to this hypothesis, the sheet of glass on which Prillieux placed the wood section served to prevent desiccation of the wood surface, but did not exclude air from the mycelium. This condition disposed the fungus to color directly on the surface of the wood.

Nutman (13), working with *Polyporus hispidus*, concluded that only old hyphae formed colored zones; and he suggested that the process resulted from the utilization of all the available nutriment in the wood. Rudau (20) reached a similar conclusion from his studies on *Fomes igniarius*. That the occurrence of colored zones often is confined to old cultures, was confirmed in the present study. The age of the mycelium, however, did not induce the formation of zones; when mycelium was exposed to the proper environmental conditions, colored zones formed, even though the hyphae were relatively young. On the other hand, when the mycelium was rapidly dried out, or was not exposed to air, zones did not form, regardless of how old the hyphae became. The formation of zones in old wood-block cultures probably was ascribable to slow desiccation of the substratum. This permitted air to enter the cellular spaces and to come in contact with the mycelium. When the entrance of air into the blocks was experimentally induced, the formation of colored zones was hastened.

Baxter (1) found that colored zones were formed by *Fomes igniarius* and *Polyporus hispidus* when these fungi were grown on wood blocks for a year. This worker attributed the phenomenon to a deposition, in various portions of the wood, of fungous decomposition products. The present investigation indicated that the zones formed by *F. igniarius* and *P. hispidus* consisted, not of decomposed wood substances, but rather of dark-colored, coarse, thick-walled hyphae. Furthermore, the zones did not occur at indefinite locations but formed where the mycelium was exposed to specific environmental conditions. In Baxter's work, zones formed only when the fungus was growing on wood or on agar substrata containing wood. He, therefore, concluded that formation of the zones was related in some way to the wood itself. In the present study, zones were occasionally formed on nutrient-agar cultures containing no wood. It seemed, therefore, that the presence of wood was not a requirement for colored-zone formation.

The factors that induced the formation of zones in agar cultures were seemingly identical with those that induced the mycelium to form zones in wood. This was found by aerating the interior of malt-agar cultures in Erlenmyer flasks, in a manner similar to that used to aerate mycelium growing in wood flour. It was observed, however, that colored zones formed less frequently on nutrient agar in Petri dishes and in culture tubes than on wood substrata. Probably the physical condition of the agar gel prevented exposure of the mycelium within the medium to the external air. The mycelium growing on the surface of the agar, on the contrary, was exposed to a large quantity of air and also was well supplied with water. It was reasonable to expect that, under these conditions, the entire surface mat of mycelium would color. Actually, when the 5 species of fungi experimented with in this investigation were grown on malt agar, the surface mycelium became brown. The subsurface mycelium, on the other hand, remained hyaline. The colored surface hyphae, especially those of *Fomes applanatus*, appeared similar to the hyphae comprising the colored zones formed in wood. The morphological

similarity between these 2 types of colored mycelia, as well as the similarity in the accompanying conditions of moisture and aeration, suggested that the phenomena were closely related. Panisset (14), working with *Daldinia concentrica* (Bolt.) Ces. et de Not., observed a similar morphological relation between the surface mycelium growing on agar and the colored-zone mycelium in the wood.

SUMMARY

An attempt was made to determine the nature and origin of the discolored zones that occurred in association with decay of the wood of *Populus canadensis* var. *eugenie* in culture by 5 species of fungi: *Fomes applanatus, F. fomentarius, F. fraxinophilus, Polyporus hispidus,* and *F. igniarius.* Microscopic study of the discolored zones showed that the phenomenon was not attributable simply to deposition or production of a pigment in the wood, but was a vital process of the hyaline type of vegetative hyphae. In the material studied, the zones consisted essentially of swollen, gnarled hyphae, associated with a brown pigment secreted by the mycelium. These zones were typical in microscopic appearance for each of the fungi studied. It was found possible to induce coloration of the hyaline type of mycelium by experimentally controlling various environmental factors. The environmental complex that stimulated coloration was the concurrent exposure of hyphae to large concentrations of water and air. This reaction occurred only in the presence of atmospheric oxygen, although the process was not one of oxidation.

Within wood, large concentrations of water and air were present concurrently only in restricted layers; hence, the pigmentation usually appeared as discolored zones or dark lines. A more extensive but similar type of coloration developed in mycelium growing on the surfaces of agar and moist wood. The following factors, through their effect on the moisture and air distribution within the substratum, had an indirect influence on coloration: (*a*) physical structure of the substratum, and (*b*) relative humidity of the air. Since pigmentation of the mycelium is a natural phenomenon resulting from definite stimuli, the vital process has been herein referred to as colored-zone formation. Coloration of the mycelium results in the discoloration of the wood; restriction of the coloration accounts for the presence of zone-lines of decay in the wood substratum.

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SEED TRANSMISSION OF MACROPHOMINA PHASEOLI

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(Accepted for publication May 18, 1938)

INTRODUCTION

The disease of beans, *Phaseolus* spp., caused by *Macrophomina phaseoli* (Maubl.) Ashby, in the United States, and commonly referred to as ashy stem blight, was, according to Kendrick (6), first observed by J. T. Barrett in California in 1919. Since there appears to have been no published record of this observation, the same disease was described in 1925 as "A new stem rot of beans" by Ludwig (7), in South Carolina, who identified the associated pycnidial fungus as *Macrophoma phaseoli* Maubl., later assigned to the genus *Macrophomina* (3).

At the time of its rediscovery in the United States, it was apprehended that the new disease might become a serious menace to the bean-growing industry. Publicity was given to it in the Science News Supplements (1) and in at least one trade journal (2). The disease was discussed by Ludwig at the Plant Disease Survey Round Table in Philadelphia in 1926. At that time he proposed the name "ashy stem blight." From year to year for the past several years since its first discovery in South Carolina, brief reports on the occurrence of the *Macrophomina* on beans, Lima beans, and cowpeas, chiefly in the southeastern States, have been filed in the records of the Divi-

sion of Mycology and Plant Disease Survey. Occasionally, local plantings would suffer severe losses induced by the fungus. In general, the reports indicated the disease to be of doubtful importance, although of wide distribution in the warmer bean-growing regions of the United States.

In 1932, Mackie (8) described damage caused by *Rhizoctonia bataticola* (Taub.) Butler in California as "a hitherto unreported disease of maize and beans." The *Rhizoctonia bataticola* and *Macrophomina phaseoli* are now commonly understood to be growth forms of the same species (5).

Kendrick (6), in 1933, and Tompkins and Gardner (9), in 1935, testify that *Rhizoctonia bataticola,* unaccompanied by the pycnidial (*Macrophomina*) stage, is capable of causing serious losses in the hot, interior valleys of California where the largest commercial plantings of beans are found. These investigators have proved definitely that pure cultures of *R. bataticola* are able to parasitize young bean plants under particular environmental conditions, the most important one of which appears to be high soil temperature.

The writer has proved that pure cultures of *Macrophomina* (with pycnidia) will, likewise, parasitize young bean plants, with symptoms distinguishable from those attributable to *Rhizoctonia* only by the presence or absence of pycnidia in advanced lesions. Data bearing upon this point, however, will be referred to sparingly in this paper. The data presented here were obtained with a quantity of southern-grown Henderson Bush Lima bean seed that proved to be infected by *Macrophomina phaseoli.*

METHODS

Sterilized (autoclaved) soil in flats was used in all greenhouse plantings and sterilized (3 minutes in HgCl, 1–1000) and unsterilized seed were planted, usually in parallel lots. The experiments extended through July, August, and early September, with daytime air temperatures usually 90° to 110° F. in the greenhouse. Temperatures fell to below 70° F. at night and ranged up to 125° F. for brief periods in full sunlight.

The greenhouse experiments were made to determine the effect of deep and shallow planting, slow and rapid germination, high and low temperatures, and high and low humidity on the course of infection, although in most cases these conditions were not under strict control.

Field plantings with nonsterilized seed were made on June 30, July 14, July 28, and August 11, using a total of 8,000 seeds in 8 12-rod rows in an overhead irrigation plot. From the standpoint of rainfall, the season was unusually favorable and only a single irrigation was made on July 10. The 2 first plantings were allowed to grow until killed by frost in October, and information was obtained on the later history of incipient stem lesions.

Formal proof that the seedling infections obtained were from infected seed consists of the following:

1. The fungus was observed on, developed on, and isolated from surface-sterilized seed.

2. The fungus developed on plants from surface-sterilized seed planted in sterilized soil.

3. No Macrophomina infections occurred on several thousand bean and Lima bean check plants grown in greenhouse and field during the same period.

Primary Infection from Diseased Seed

Kendrick (6), Tompkins and Gardner (9), and Dastur (4), in studies on beans, cowpeas, and cotton, all emphasize the importance of the cotyledons in primary infections by *Macrophomina phaseoli* (or *Rhizoctonia bataticola*). With the fungus established in the cotyledon, it seems unimportant whether the infection of the seed comes after it is planted or before it is harvested, since in all likelihood the infection of seed in the pod would occur through contact of the pod with infested soil. The occurrence of natural seed transmission, nevertheless, may be an important circumstance in accounting for the occasional serious outbreaks of the disease in regions where home-grown seed is apt to be used. Furthermore, in the writer's experiments, consistently more seedling infection is obtained when the seed is infected before planting than when infection is depended upon to occur from inoculum in the soil. The fungus in a pre-infected seed may have the advantage of no more than a day's growth; yet, even a few hours difference in the incubation period may affect the amount of damage caused to seedlings by the *Macrophomina*, which grows with great rapidity at high temperatures and on a favorable substrate.

The artificial infection of bean seed by pure cultures of *Macrophomina* has been accomplished repeatedly, and a higher percentage of seedling mortality is obtained from such pre-infected seed than from healthy seed planted in heavily infested soil.

The first direct evidence of natural seed infection by *Macrophomina* was obtained in September, 1935, when pycnidia of the fungus were found on a few mature pods in the experimental plots at Beltsville, Maryland. The pycnidia were numerous on the exterior of the pods and contained normal *Macrophomina* spores. The seeds also were obviously infected but bore no pycnidia. Isolations from the surface-sterilized seed coats and from the cotyledons all gave sclerotial cultures of *Macrophomina*.

During the summer of 1937 it was possible to demonstrate that seed transmission of *Macrophomina phaseoli* may be of economic importance. With the cooperation of W. D. Moore, about 10 lbs. of home-grown Henderson Bush Lima bean seed were obtained from a farmer near Tifton, Georgia, who had planted 20 acres with seed of the same source and had obtained only a 5 per cent stand.[1] The principal cause of the damage was identified by Dr. Moore as *M. phaseoli*, and specimens of diseased seedlings collected 12 to 16 days after planting bore symptoms resembling those described by Kendrick (6) and Tompkins and Gardner (9) on bean and cowpea seed-

[1] The writer wishes to express gratitude to Dr. Moore for his generous cooperation in this work.

lings, and identical also with those obtained by the writer on bean seedlings with pure cultures of *M. phaseoli*. Pycnidia were abundant on the blackened stems. As in all similar material, the lesions extended predominantly from the cotyledonary node.

Many of the seeds were visibly infected by *Macrophomina*. Numerous small, black sclerotia beneath the seed coat gave an ashy-grey appearance to the exterior of the seed. Laboratory germination tests were made on moist blotting paper, using seed, surface-sterilized 3 minutes with 1–1000 mercuric chloride. *Macrophomina* developed on 57½ per cent of the seed, or 212 out of 369. Only 31 per cent germinated, principally because of the rapid decay caused by *Macrophomina*. Pycnidia developed on most of the infected seed but isolations from these gave only sclerotial cultures of *Macrophomina*. Seed from the same lot was used in a series of greenhouse and field-planting experiments made at intervals of 1 to 3 weeks, most of the results of which are shown in tables 1 and 2.

The total amount and character of injury to plants developing from the infected seed varied greatly in plantings made at several different times and under different conditions. Table 1 shows that in 5 lots of 500 seeds each, planted at intervals of 1 to 3 weeks, there was a regular increase in percentage germination and a regular decline in the percentage of plants infected. These results do not correlate satisfactorily with greenhouse temperature records. If, however, comparison be reduced to the 2 extremes (July 1 and August 25), there seems to be correlation between temperature and infection. In figure 1, A, the 10-day infection period is divided into 2 phases, the preemergence phase and the postemergence phase. It is possible that temperature exerts an opposite effect in these 2 phases of the infection period; if so, the curves in figure 1, A, could be interpreted as follows: Higher temperature in the preemergence phase favors rapid embryo development and reduces seed decay, whereas higher temperature in the postemergence phase may favor the pathogen (with an optimum of *ca* 35° C.) more than the host.

In similar data from field tests (Table 2 and Figure 1, B) the lot with higher temperature in the germination period also gave the highest percentage germination and lowest percentage infection.

These data are somewhat contrary to the results of Kendrick (6) and Tompkins and Gardner (9), who found that high soil temperatures favored infection by the sclerotial form of the pathogen. The discrepancy may mean either that temperature is not the principal controlling factor in the disease or that the actual temperature level at Beltsville was too low to reveal the maximum effect of temperature on the pathogen. Data were not available on soil temperatures in the Beltsville experiments.

Data in table 1 show that surface-sterilizing the infected seed consistently reduced the percentage of plants infected by approximately one half. The nonsterilized series shows the same seasonal increase in percentage germination and decrease in percentage infection as occurred in the sterilized series.

TABLE 1.—*Greenhouse Series: Relation of date of planting and seed treatment to development of Macrophomina phaseoli on Henderson Bush Lima bean seedlings from naturally infected seed planted in the greenhouse in 1937*

Date of planting	Seed treatment	No. seed planted	No. plants emerged	Percentage emerged	No. of emerged plants infected	Percentage emerged plants infected	Percentage leaves infected
July 1	Sterilized	500	297	59.4	88	29.6	.....
July 21	"	500	274	54.8	80	29.2	.....
Aug. 4	"	500	313	62.6	69	21.8	.....
Aug. 25	"	500	355	71.0	41	11.5	.....
Aug. 18	Sterilized + high humidity	500	336	67.2	5	1.5	49.4
July 21	Unsterilized	500	163	32.6	99	61.0	.....
Aug. 4	"	500	288	57.6	172	59.7	.....
Aug. 25	"	500	335	67.0	103	30.7	.....
Aug. 25	"	500	347	69.4	109	31.4	.....
July 21	Sterilized + deep planted	500	229	45.8	36	15.7	.....
Aug. 18	Unsterilized + high humidity	500	338	67.6	30	8.8	59.1

TABLE 2.—*Field Series: Relation of temperature to development of Macrophomina phaseoli on Henderson Bush Lima bean seedlings from naturally infected seed planted in the field at Beltsville, Maryland, in 1937*

Date planted	Seed treatment	Seed planted	Av. daily mean temp. in °F. for 10 days after planting	Av. daily mean temp. of preemergence phase	Av. daily mean temp. of postemergence phase	No. of plants emerged	No. of emerged plants infected	Percentage germination	Percentage infection
June 30	None	2000	73.6	69.4	77.8	693	366	34.6	52.8
July 14	None	2000	81.5	82.8	80.2	1041	168	52.0	16.1
July 28	None	2000	74.1	74.4	73.8	711+[a]	228±	35.5+	32.0±
Aug. 11	None	2000	.....	.....	77.8	823	307	41.1	37.5

[a] Results of this test were complicated by worm infestation.

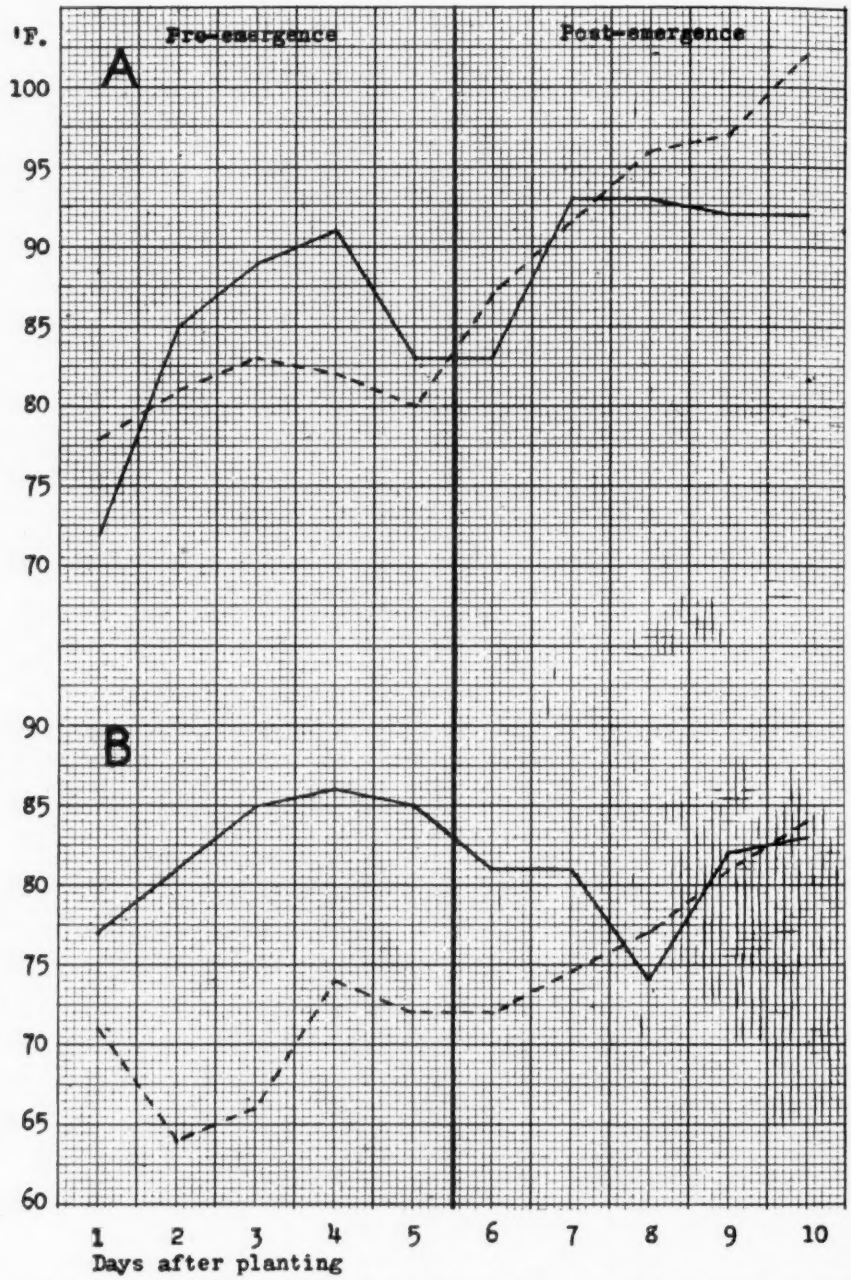

FIG. 1. Correlation between temperature and seed borne-Macrophomina infection. A. In greenhouse: broken line, low germination and high infection (July 1 to 10); solid line, high germination and low infection (Aug. 25 to Sept. 3). B. In the field: broken line, low germination and high infection (June 30 to July 9); solid line, high germination and low infection (July 14 to 23).

It might be supposed that the pathogen merely declined in viability in the seed were it not for the fact that field data do not show the same seasonal change.

The 2 lots planted August 18 (Table 1) show the remarkable effect of rapid germination and rapid emergence. These lots were kept in glass-covered infection chambers for 7 days after planting. Seedlings began to emerge on the second day after planting because of the higher temperature and especially favorable moisture conditions. Seedlings elongated rapidly and became extremely etiolated. On the 5th day, cloudy and cold weather set in. Very few infections occurred at the cotyledonary node, but a high percentage of primary leaves were infected as a result of contact with infected cotyledons accompanied by excess moisture. Pycnidia developed on necrotic portions of the infected leaves. When the plants were removed from the humidity chambers, development of the lesions ceased abruptly. When returned to the humidity chambers, the lesions resumed development and in several cases the infection progressed through the petiole into the stem and produced ashy-stem-blight symptoms.

The seedling phase of Macrophomina infection is definitely distinguishable from the disease on older plants. The term "ashy stem blight" originally referred to older plant infections where an "ashy" color was imparted to stems and petioles by the presence of numerous pycnidia underneath the translucent epidermal layer. The seedling phase is a type of damping off in which the term "charcoal rot," used by Tompkins and Gardner (9) and others, adequately describes the deep black lesions. Pycnidia never developed on the material used by Tompkins and Gardner, while, in the present case, they developed externally in advanced lesions and subepidermally only on older plants. The ashy appearance is thus absent in the seedling phase.

Typical infection occurs from the cotyledonary node (Fig. 2). Once established beneath the seed coat, the fungus is able to penetrate the stem through the base of the cotyledon. A few instances were observed where infection had begun at the tip of the plumule and on the main root, probably from contact with the infected seed coat during germination. In the former instances the primary leaf became completely blasted and the fungus proceeded through the petiole into the stem.

Apparently, some degree of infection of the seedling is essentially assured, provided the fungus becomes established in the seed coat before germination. Conditions favoring the adherence of the infected seed coat to the cotyledon probably will favor infection. Likewise, conditions favoring adherence of the cotyledons to the stem will favor stem invasion. Infected cotyledons frequently adhere to the stem long after the noninfected cotyledons have dropped off, due possibly to the presence of mycelium at the point where abscission would normally occur.

Death of the seedling seems to result when the fungus invades the stem before the seedling is completely emerged from the soil. If invasion of the stem is delayed beyond that stage, only an incipient lesion develops at the

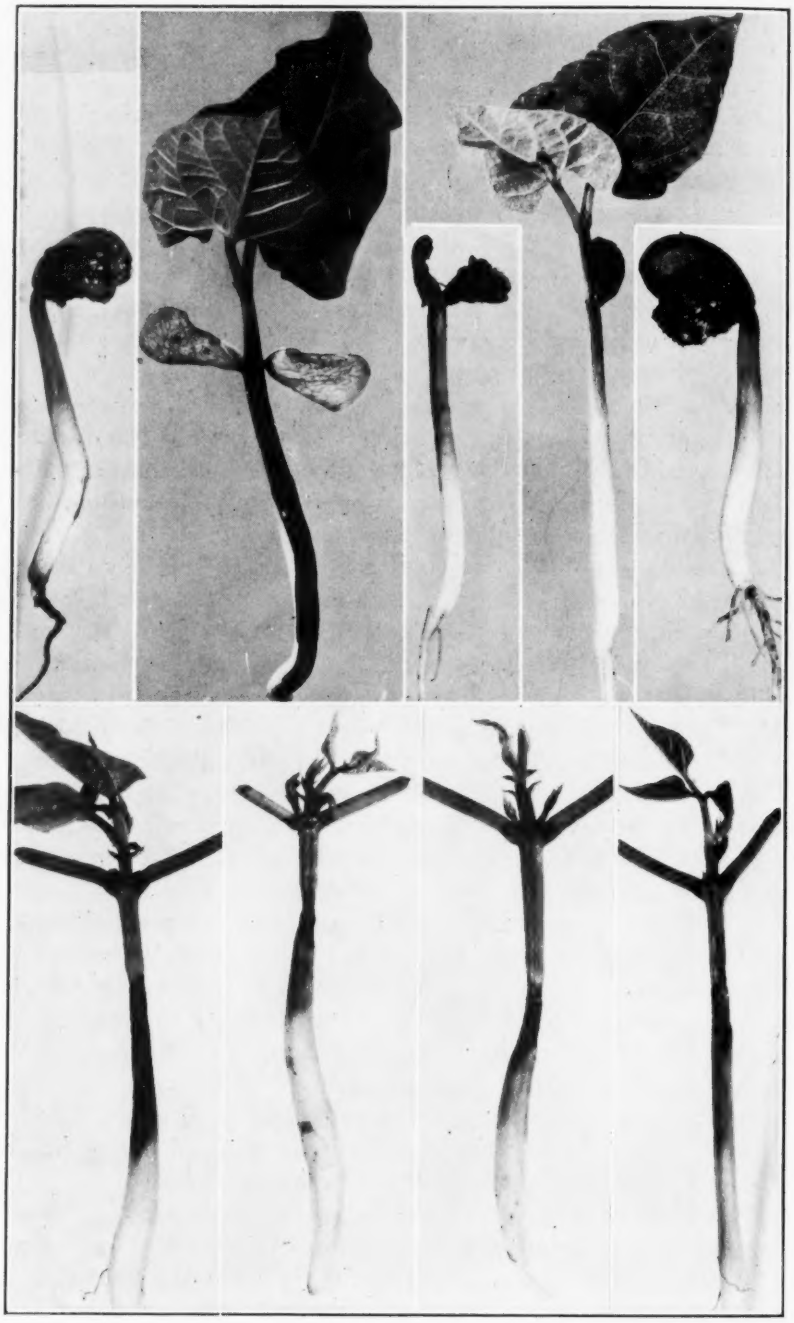

FIG. 2. Seed-borne *Macrophomina phaseoli* infection of Henderson Bush Lima in the greenhouse showing degrees of seedling injury 7 days after planting (above) and 20 days after planting (below). Approximately natural size. Photographs by Guernsey.

cotyledonary node or the infected cotyledon is cast off before the fungus reaches the stem and no stem infection results. Apparently, any condition, either hereditary or environmental, that prolongs the germination period will favor seedling infection unless it has, at the same time, a sufficiently depressing effect upon the parasite.

Some results indicate that deep planting of infected seed increases the number of seedlings killed prior to emergence, but does not increase the total number of infected plants (Table 1). When noninfected seed is planted in inoculated soil, deep planting probably would increase the total percentage of infection because of the longer time required for the plants to emerge above the soil.

Tompkins and Gardner (9) considered the relative freedom from infection by *Rhizoctonia bataticola* of cowpeas as compared with beans at high temperatures as attributable in part to their more prompt germination and more rapid hypocotyl elongation. Also, Dastur (4), in reference to *Macrophomina phaseoli* infection of cotton seedlings, states, "Any delay in the appearance of the cotyledons above the soil surface favors infection of the seedling. Seeds that are naturally very slow in sprouting are particularly susceptible; and, where soil conditions themselves are unfavorable to quick germination, heavy losses may occur."

Under usual conditions of growth, the parasitism of the fungus on beans seems strictly limited to the seedling host. Progress of a lesion is stopped abruptly after the first few days growth of the plant. Thus, on many plants, incipient lesions are observed in the cotyledonary scar, where penetration of the stem by the fungus was for some reason delayed (Fig. 3). The incipient lesions ordinarily make no further growth during the lifetime of the plant, although they may be extensive enough to cause breaking at various stages before the plant reaches maturity. Growth of callus tissue within the dormant lesion sometimes obliterates evidence of seedling infection.

Secondary Infection

It has been pointed out that the primary infection of the seedling from infected seed ceases its development abruptly during the first few days after emergence of the seedling aboveground. Stem lesions may remain throughout the life of the plant without undergoing any appreciable further growth (Figs. 3 and 4). At the same time, it is well known that infections do occur on older plants, giving rise to the ashy-stem-blight phase of the disease.

Attempts to produce older plant infections in the greenhouse have been successful only under conditions of prolonged high humidity, and in the few instances where ashy-stem-blight symptoms resulted (Figs. 4 and 5), the infections seemed to have extended from local secondary lesions on leaves and petioles.

Reference already has been made to the occurrence of primary leaf infec-

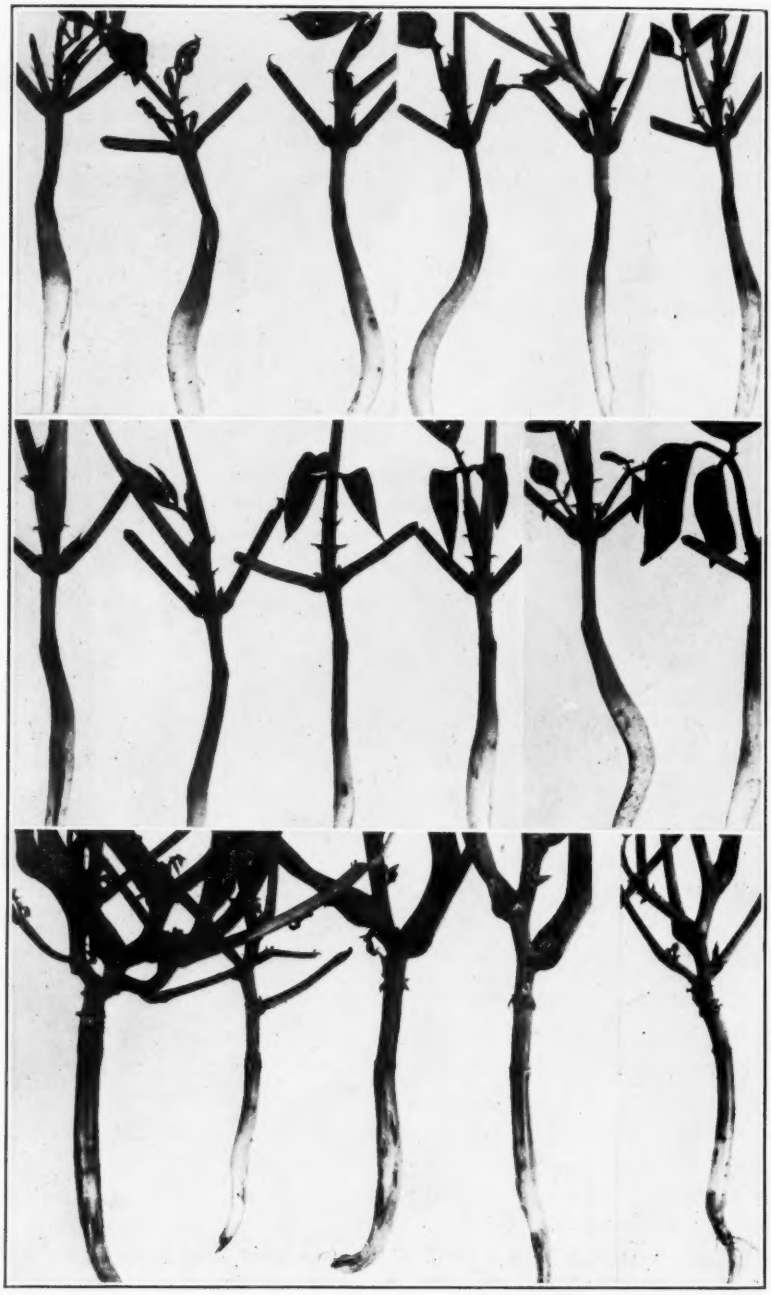

FIG. 3. Seed-borne *Macrophomina phaseoli* infection of Henderson Bush Lima in the field, showing restricted (above) and incipient (center) lesions at the cotyledonary node 20 days after planting. Below are incipient lesions that have made no further growth in an additional 4 weeks. *Ca.* $\frac{1}{2}$ to $\frac{2}{3}$ natural size.

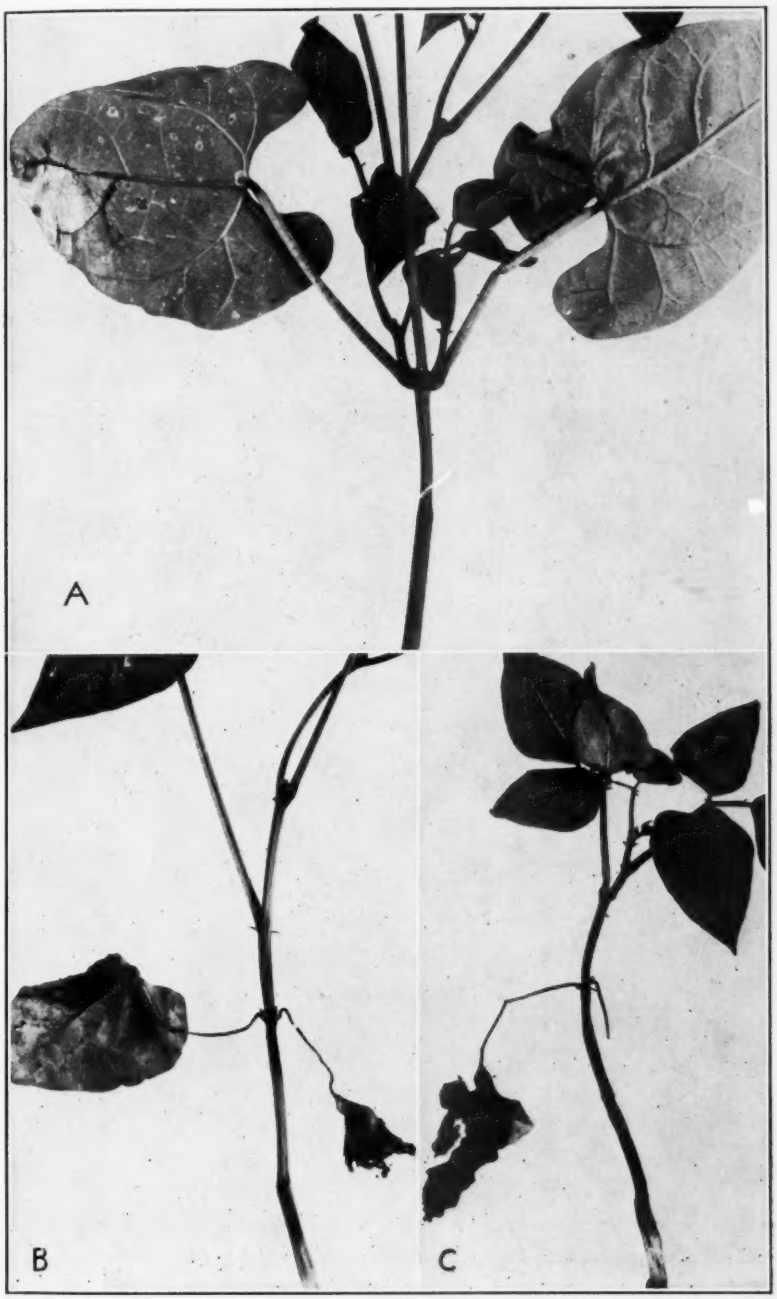

FIG. 4. *Macrophomina phaseoli* on Henderson Bush Lima beans 4 and 5 weeks old. A. Undeveloped primary infection at the cotyledonary node and secondary infection of a primary leaf. B. Primary infection at the cotyledonary node and secondary stem invasion at the primary leaf node. C. Advanced primary and secondary infection with abundant pycnidia on leaf, petiole, and stem. *Ca.* $\frac{1}{2}$ to $\frac{2}{3}$ natural size.

FIG. 5. *Macrophomina phaseoli* on Henderson Bush Lima aged 7 weeks. Primary infection at the cotyledonary node has remained dormant, while secondary infection from the primary leaf has advanced down the stem, producing ashy stem blight. *Ca.* $\frac{1}{2}$ to $\frac{3}{5}$ natural size.

tion of beans as a result of contact with infected seed coats at the time of germination. Artificial infections also have been produced on leaves of Henderson Bush Lima by inoculating with pure cultures of a pycnidial strain on agar. If held for a sufficiently long time at relatively high humidity, a leaf infection progresses through the petiole and into the stem. Pycnidia form on the decayed leaves and petioles and in the more extensive stem lesions (Figs. 4 and 5).

The most abundant pycnidium formation and most rapid development of the fungus are favored by continuous high humidity. When the infected plants are removed to a relatively dry atmosphere, the lesions make almost no growth. When restored again to the humid situation the lesions resume development.

With the combination of leaf infection and prolonged periods of high humidity and relatively high temperature, it is possible to account for the occasional severe outbreaks of ashy stem blight under field conditions in the southeastern United States. It will be necessary to make an extensive field study of the disease before the conditions favoring natural infection of older plants can be determined accurately, yet it is the impression of some who have observed the disease in the South that it develops more extensively during wet seasons.

SUMMARY

The occurrence of natural seed transmission of *Macrophomina phaseoli* is demonstrated in Henderson Bush Lima bean seed grown in Georgia. The amount of infection approximated 85 per cent in nonsterilized seed and 57 per cent in surface-sterilized seed, thus indicating that the fungus was established beneath the seed coat in over half the seed. Sterilization of seed before planting reduced subsequent seedling infection by approximately 45 per cent.

Amount of seedling injury developing from infected seed is inversely correlated with percentage of germination. Factors operating during the germination period seem to be the most important in determining the amount and extent of primary infection. Apparent correlation between temperature and infection point mainly toward its effect on germination.

Humidity is a controlling factor in secondary infection of leaves, and prolonged high humidity was necessary to produce symptoms of ashy stem blight on older plants.

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ANNUAL GROWTH[1] RATE OF CRONARTIUM RIBICOLA CANKERS ON BRANCHES OF PINUS MONTICOLA IN NORTHERN IDAHO

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(Accepted for publication April 25, 1938)

INTRODUCTION

Early studies of white pine blister rust (*Cronartium ribicola* Fisch.) on western white pine, *Pinus monticola* Dougl., were concerned chiefly with determining to what extent trees of that species might be damaged by the disease. From these studies it became apparent that, except in pole-size and smaller trees growing under conditions exceptionally favorable to the rust, damage almost always would result from trunk girdling rather than from multiple twig and branch infections[3] (1; 4, p. 883; 5, p. 245; 6, p. 491). The next phase of the studies was an attempt to predict the probable extent of damage to pines and the period required for such damage to be realized. These predictions were necessarily based on infections already present in which the hyphae of *C. ribicola* would grow down the branches of the host tree and enter and girdle the trunk (1, 2, 7). Before such predictions could be made with any reasonable degree of accuracy a knowledge of the rate of promixal advance of the mycelium was necessary. Lachmund (6) quite fully summarized the data on the growth of blister-rust cankers on western white pine in British Columbia. Similar but less extensive studies dealing with the proximal growth of branch cankers have been more recently conducted in northern Idaho and the pertinent data secured are herein presented.

SELECTION OF CANKERS FOR GROWTH MEASUREMENTS

In selecting cankers for measurement the principal consideration was to obtain cankers on as wide a range of branch diameters as possible, it having

[1] The term "growth" of canker is used to define the elongation of the swollen and discolored area of bark that accompanies and bears a quite constant relationship to the progress of the fungus mycelium.

[2] The author is indebted to J. L. Mielke of the Division of Forest Pathology and to H. G. Lachmund, formerly of the same Division, for initiating the work on several of the study areas. Acknowledgements also are extended to those various members of the Division, both present and former, who assisted in the routine collection of data.

[3] Childs, Thomas W. and J. W. Kimmey. Studies on probable damage by blister rust in some representative stands of young western white pine. Jour. Agr. Research. (In press.) 1938.

been previously demonstrated that canker growth varies directly with the size of infected branch (6, p. 479; 8, p. 517). The numerical basis employed in each diameter class was roughly proportional to the natural frequency of cankers as found in the field. The second most important consideration was to choose cankers representing a wide range of developmental stages. This was to compensate for any variations in rate of growth that might have been traceable to differences in the length of time the fungus had been in the bark. All cankers selected were on trees ranging from 3 to 30 feet in height and averaging 12 to 20 feet. These canker-bearing trees were from typical stands of reproduction and were selected at random in so far as possible to do so and still secure trees bearing cankers from which a year's growth measurement could be obtained. In selecting canker-bearing branches only those were rejected that obviously would die from suppression before a year's growth could be secured on the canker. The ability of the investigator to reach a cankered branch from the ground was the only other factor influencing the position in the tree of the branches selected. Since several relatively small trees were available, however, it was possible to sample cankers in all portions of the crown, from the extreme top to the very base.

At intervals between the fall of 1932 and the summer of 1936, 18 series of cankers were marked for growth measurement in northern Idaho. These series embraced 666 cankers on a total of 167 trees at 9 different localities. On many of these cankers it was possible to secure two consecutive annual-growth measurements, but, in other cases, cankers and even entire trees and series had to be omitted for reasons enumerated in the following section. Upon the completion of the study in 1937 a total of 476 measurements of annual canker growth were available for analysis. These cankers were on 103 different trees from 15 of the original series at 6 localities. These 6 localities were all in the St. Joe region, well within the commercial white pine belt, at elevations between approximately 2500 and 3500 feet.

Table 1 presents a summary of the bases for this study and also shows the dates on which the cankers were first marked as well as those on which the initial and final growth data were taken.

MARKING AND MEASURING CANKERS

The limits of discoloration of the infected bark were used as indicating the extremities of the canker on the branch. Colley (3, p. 625) states in connection with his studies on blister rust on eastern white pine, *Pinus strobus* L., that "the irregular edge of the etiolated area marks fairly definitely the advancing tips of the invading hyphae, which generally extend a little beyond the line." Opie,[4] working with western white pine, found a positive correlation between the diameter of the canker and the extent of advance of the mycelium beyond the visible limits of discoloration.

It is evident, then, that using the limits of discoloration as the actual

[4] Opie, Robert. The relationship of *Cronarthium ribicola* to *Pinus monticola*. Unpublished Master's thesis. Univ. of Idaho. 1937.

TABLE 1.—*Bases used and important dates in the study of annual proximal growth of Cronartium ribicola cankers on branches of Pinus monticola in northern Idaho*

Localities	Basis			Cankers marked	Measurement period for annual growth	
	Trees	Cankers	Measurements annual canker growth		Beginning	End
	(*No.*)	(*No.*)	(*No.*)	(*Date*)	(*Date*)	(*Date*)
Deep Creek—Elk River	6	25	25	Sept. 16, 1932	July 11, 1933	July 19,1934
St. Maries River—Gold Center Creek						
Series 1	5	17	17	Sept. 13, 1932	June 6, 1933	June 14, 1934
			12		May 17, 1934	May 23, 1935
Series 2	5	22	22	Sept. 13, 1932	June 6, 1933	June 14, 1934
			13		May 17, 1934	May 23, 1935
Ruby Creek						
Series 1	16	31	31	Sept. 15, 1932	July 10, 1933	July 20, 1934
			20		May 15, 1934	May 22, 1935
Series 2	8	43	43	Oct. 5, 1933	May 15, 1934	May 22, 1935
Camp 45	25	44	44	Aug. 28, 1933	Aug. 28, 1933	Aug. 18, 1934
			32		Sept. 18, 1934	Sept. 20, 1935
Crystal Creek						
Series 1	12	38	38	July 5, 1933	Oct. 6, 1933	Oct. 10, 1934
			31		Sept. 19, 1934	Sept. 20, 1935
Series 2	14	51	51	July 6, 1933	Oct. 6, 1933	Oct. 10, 1934
			37		Sept. 19, 1934	Sept. 20, 1935
Emerald Creek	12	60	60	July 23, 1936	July 23, 1936	July 20, 1937

limits of the canker did not introduce any significant error in this canker growth study. If, for example, the hyphae extended .5 inch beyond the visible limits of discoloration on a given canker at the time of marking they should still be approximately .5 inch beyond the new limit of discoloration at the time of measurement one year later.

The limits of proximal discoloration were marked by a narrow band of bright yellow paint. The diameter of the branch at this point was measured to the nearest .01 inch. All subsequent records on the growth of the canker down the branch were secured by measuring from the original paint mark to the new extremity of discoloration. At the time of each periodic remeasurement of growth the branch diameter at the limits of discoloration was recorded. This provided the new basic diameter in the event that successive annual growth measurements could be secured on a given canker. With only one series of cankers did a measurement date fall even approximately one year from the date of marking. In all other cases it was necessary, for the sake of accuracy, to compute annual growth as between the two measurement dates that fell most nearly one year apart.

Growth measurements were not secured from every canker originally marked. The causes of loss of cankers from the basis, as listed below in order of their importance, are shown because many of the losses in this study were due to factors that need to be reckoned with in applying these results to studies of pine damage.

1. Failure to measure the canker at the proper intervals to give even approximately one year between any two measurements.
2. Destruction of trees in clearing rights-of-way.
3. Gnawing of cankers by rodents.
4. Parasitism of the cankers by secondary fungi.
5. Coalescence of cankers.
6. Killing of the host branch by the canker.

These losses in numbers were partially offset by measurements for 2 successive years on other cankers.

RESULTS

The 476 annual proximal growth measurements were summarized by branch diameter classes. Branch diameter at the base of the discolored area at the beginning of the growth measurement period was the basis for segregation. All 12 diameter classes between .01–.10 and 1.11–1.20 inches, inclusive, were represented.

Table 2 shows the basis for each branch diameter class and the average proximal canker growth over a period of a year. The standard deviation from the average growth is given for all diameter classes having a basis of 25 or more cankers.

From the basic data the regression equation for annual proximal growth over branch diameter was computed on the assumption that the data best fitted a straight-line formula. This formula was determined as being

TABLE 2.—*Average annual proximal growth of Cronartium ribicola cankers on Pinus monticola branches of various diameters in northern Idaho*

Diameter of branch		Basis				Annual proximal growth	
Range	Average	Areas	Series	Trees	Canker measurements	Average	Standard deviation
(*In.*)	(*In.*)	(*No.*)	(*No.*)	(*No.*)	(*No.*)	(*In.*)	(*In.*)
.01– .10	.10	1	1	1	2	1.38	.....
.11– .20	.18	4	12	35	83	1.36	±.34
.21– .30	.26	6	15	76	164	1.44	±.34
.31– .40	.35	6	15	58	93	1.52	±.38
.41– .50	.46	6	12	35	58	1.68	±.44
.51– .60	.56	6	12	19	25	1.84	±.50
.61– .70	.65	3	5	12	15	1.85	.....
.71– .80	.76	3	4	10	15	1.84	.....
.81– .90	.84	2	4	6	10	2.12	.....
.91–1.00	.95	2	2	5	6	2.32	.....
1.01–1.10	1.08	2	2	3	3	2.21	.....
1.11–1.20	1.13	2	2	2	2	2.41	.....
Totals		6	15	103	476		

$Y = 1.15 + 1.116X$. In figure 1 this formula is plotted to permit reading annual proximal canker growth on branches of any size between .10 and 1.20 inches in diameter.

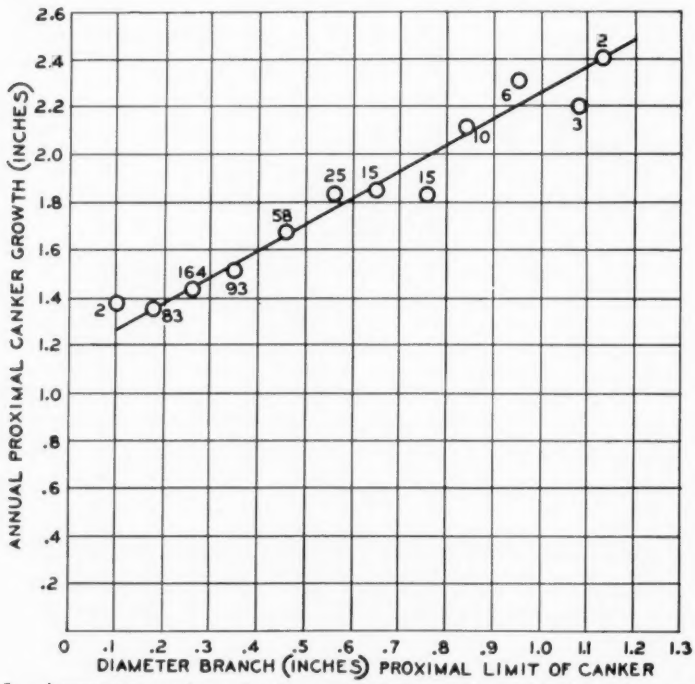

FIG. 1. Average annual proximal growth of *Cronartium ribicola* cankers on branches of *Pinus monticola* in northern Idaho. Data points are actual average values by branch diameter classes. Numeral beside each point shows the number of cankers used as a basis in determining its location.

The coefficient of correlation between canker growth and branch diameter was found to be 0.477 ± 0.035. From this figure it would seem that there are factors other than branch diameter exerting considerable influence on proximal canker growth. Branch diameter, however, is highly significant and, even when used alone, is probably satisfactory for studies whose results are desired for field application. Certainly, branch diameter is more readily measured in the field than any of the other possible influencing factors.

DISCUSSION

The number of factors that might possibly exert an influence on the growth of any individual canker are almost innumerable. The data secured have been analyzed on what has been previously found to be the most influential factor—diameter of infected branch.

The possible influence of certain of the remaining factors has been analyzed where a sufficient basis was available. In table 3 it is shown that with increasing age of the canker there is an apparent acceleration of the growth rate. A definite statement to this effect is not justifiable on the relatively small and heterogeneous basis available. It would seem justifiable, however, to conclude that the growth rate does not become less with increasing age up to at least the second season of aecial production. In this analysis cankers from all areas were used but to eliminate seasonal variation in rate of growth only cankers measured during 1933–34 were included.

TABLE 3.—*Influence of age of canker on average annual proximal growth of Cronartium ribicola cankers on branches of Pinus monticola in northern Idaho*

Diameter class	Age of canker								
	Incipients and juveniles			First and second pycnia			First and second aecia		
	Basis	Diameter	Growth	Basis	Diameter	Growth	Basis	Diameter	Growth
(*In.*)	(*No.*)	(*In.*)	(*In.*)	(*No.*)	(*In.*)	(*In.*)	(*No.*)	(*In.*)	(*In.*)
.11–.20	15	.16	1.38	30	.18	1.52	6	.19	1.61
.21–.30	23	.26	1.44	37	.26	1.63	21	.27	1.64
.31–.40	12	.35	1.52	8	.34	1.70	21	.36	1.77
.41–.50	4	.42	1.71	9	.44	1.55	18	.47	2.00

A similar analysis was made of 113 flagged[5] and 211 nonflagged cankers. No consistent superiority in proximal growth was noted for either type of canker. This is in direct accord with Lachmund's (6) findings in British Columbia. Averaging all diameters together the flagged cankers grew 1.49 inches proximally, while the nonflagged cankers were growing 1.52 inches. The average diameter of branch was .32 inch in each case. In this analysis data for a given diameter class and from a given area were not used for nonflagged cankers unless flagged cankers were available for direct compari-

[5] Cankers that have caused the death of the foliage out to the tip of their supporting branch (distally).

son and *vice versa*. It must be borne in mind, however, that none of the cankers measured were flagged at the beginning of the study. All flagged cankers suffered death of their distal ends during the course of the study.

Although the effect of age of canker and flagging have been analyzed separately, cankers of all ages and flagged cankers were included in the data on which table 2 and figure 1 are based. Obviously, these two variables did not appreciably alter the reliability of the results. The remaining factors, which might exert an influence on the rate of growth, are more or less incapable of quantitative expression. The procedure of taking a large sample over a wide range of environment, however, served largely to eliminate them from consideration in the practical application of the results. The influence of site was at least partially eliminated by marking cankers on trees from 6 distinct localities. Marking cankers on 103 different trees and an even greater number of individual branches tended to average out possible variations in rate of growth because of the vigor of the host. Possible variations in canker growth due to gross annual climatic differences were largely eliminated by taking annual measurements over 3 distinct periods; 1933–34, 1934–35, and 1936–37. The data indicated, however, that the period 1933–34 was decidedly more favorable for canker growth than was 1934–35. Direct comparison was not possible for the period 1936–37. By starting at least one series of annual-growth measurements during each of the months from May until October, possible variations in seasonal rate of growth were largely eliminated. Cankers that had grown through nodes were included as well as cankers that developed throughout the year on one internode only. Cankers suffering slight chewing by rodents were included, but severely chewed cankers were discarded.

These data, then, are presented as representing a general cross-section of the proximal growth that blister-rust cankers may be expected to make on branches of western white pine in Idaho. Although data are presented for branch diameters up to but 1.20 inches, branches of this and even smaller sizes occur abundantly in pole and even mature trees of this species. These canker-growth figures are considered to be directly applicable in studies of damage to western white pine trees up to at least 30 feet in height. For larger trees it is hard to conceive of the application of these growth figures resulting in significant errors when estimating time before damage, since, in damage studies, estimates of time that fall within ± 5 years of the actual are generally sufficiently accurate.

Lachmund's publication on canker growth (6, p. 491–499) should be carefully read before attempting to apply these growth data to studies of pine damage. In that publication the methods of application are given in detail and precautions to be taken are discussed. It must be borne in mind, however, that the average proximal canker growth in Idaho is comparable only to the growth found for the least favorable areas measured in British Columbia.

SUMMARY

During the years 1933 to 1937 annual proximal growth measurements were secured on white-pine blister-rust cankers growing on branches of western white pine within its commercial range in northern Idaho. Upon the completion of the study 476 measurements were available for summarization. This total was secured from 15 series of annual growth measurements on 103 trees from 6 different localities.

These data were segregated into branch-diameter classes for analysis, it having been previously shown that canker growth varies directly with the size of infected branch. All diameter classes between .01–.10 and 1.11–1.20 inches were represented.

The growth of cankers on branches of various sizes is shown graphically. This graph shows a straight-line trend, the growth varying from 1.26 inches on branches .10 inches in diameter to 2.49 inches on branches 1.20 inches in diameter. Growth of cankers on branches of any intermediate size can be read directly from the curve. In addition it was determined that flagging and age had no significant effect on the proximal growth of branch cankers although it appears there may possibly be some increase in rate of growth accompanying an increase in age of cankers. Other factors, incapable of quantitative expression but that perhaps influence canker growth, are discussed.

These results are considered to be of direct application in conducting pine damage studies on western white pines up to 30 feet in height. For still larger trees the application of these data should not result in significant errors.

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LEAF BLIGHT OF IRIS CAUSED BY BACTERIUM TARDICRESCENS

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(Accepted for publication May 12, 1938)

INTRODUCTION

A bacterial disease of iris leaves, which appears to be increasing in severity and distribution, has been under observation by the writer since 1924, when some diseased leaves were received from Virginia. Specimens have continued to come to the Department of Agriculture each season, but beyond determining that it was caused by bacteria, not much attention was given to the disease.

From 1934 to 1936, a number of seriously infected plants were received from Virginia, Maryland, and the District of Columbia. Most of the specimens have been sent in by growers in or near the District of Columbia, but other regions, from Alabama to Massachusetts, have been represented. All the specimens received to date (January, 1938) are varieties of the bearded group with one exception. This is *Iris cristata*, from Connecticut, sent in by the late Dr. G. P. Clinton, in 1933.

It is not likely that this is a new disease, but it is probable that the weather conditions of the past few years may have particularly favored its development in certain regions, and that observant growers are distinguishing it more frequently from other iris diseases. This disease was reported and the causal organism named and briefly described by the writer[1] in 1936. Later, Burkholder,[2] also, described it.

Except for these two reports, no record has been found in plant pathological literature of a similar iris disease. In 1931, Takimoto[3] in Japan described a bacterial leaf spot of iris and named the causal organism *Bacterium iridicola*. He sent cultures of this organism and specimens of infected iris leaves to the writer. A study of these showed that the leaf lesions and the character of the organism were unlike those of *Bacterium tardicrescens*. His description also was of an organism quite unlike *Bact. tardicrescens*.

DESCRIPTION OF THE DISEASE

Usually the first observed indications of the disease are the rather large, dark green, water-soaked spots on the leaves (Fig. 1, A). These areas are conspicuous in early morning or after any period of moist weather. A few hours of even ordinary dry weather cause a large part or even all of these areas to disappear, leaving only small, yellowish-green spots. With a renewal of moist conditions, the dark, water-soaked areas reappear.

[1] McCulloch, Lucia. An iris disease caused by *Bacterium tardicrescens*, n. sp. Paper read at the 28th Meeting of The American Phytopathological Society, Atlantic City, N. J., Dec. 28–31, 1936.

[2] Burkholder, W. H. A bacterial leaf blight of iris. Phytopath. **27**: 613. 1937.

[3] Takimoto, S. Bacterial leaf spot of iris. Fungi (Nippon Fungilogical Soc.) **1**: 21–24, 1931 (In Japanese with English Summary). [Abstract in Rev. Appl. Mycol. **11**: 108. 1932.]

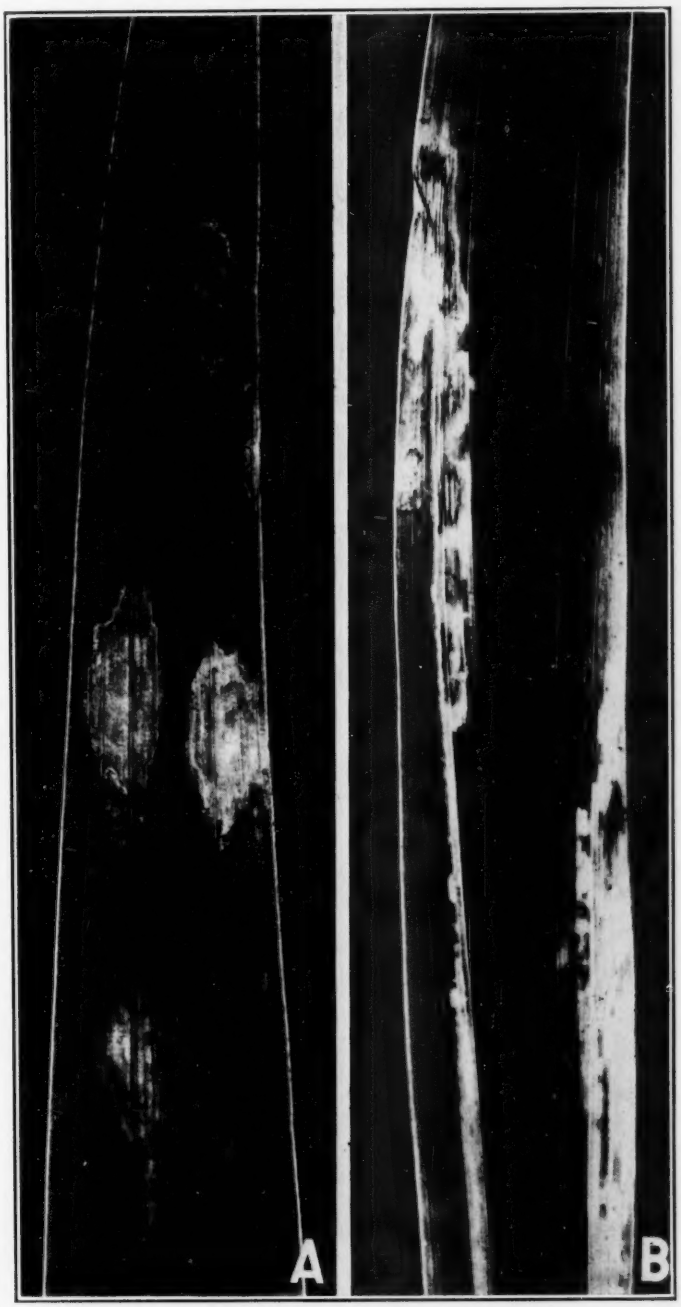

FIG. 1. Bacterial leaf blight of iris. A. Natural infection. Recent lesions with wide, water-soaked margins and isolated, water-soaked spots, photographed by transmitted light. B. Natural infection. Old lesions, dry and collapsed in the centers, photographed by reflected light. Slightly enlarged.

FIG. 2. Iris bacterial leaf blight. Variety magnifica. Inoculated May 27, 1936. These four leaves are from one plant. Photographed on June 8, 1936. × approximately 1.

Streaks 1 to 6 inches long of fairly uniform width and large, irregular spots are common (Fig. 2). In early stages these vary in size and shape with alterations in atmospheric humidity.

Lesions occur on all parts of the leaves above the extreme base, but they are most frequently found on the margins. Tiny, pale spots, at first visible on one side only, enlarge, become translucent, and extend through the leaf. In a dry, or even moderately dry atmosphere the spots increase in size slowly or not at all and the water-soaked margins are lacking. In such cases the infection may be unnoticed or mistaken for the common leaf spot caused by *Didymellina macrospora* Kleb. The bacteria remain alive, though inactive, as long as the leaf lives, and, in any period of sufficient atmospheric humidity, they renew growth and produce the characteristic lesions. From large active lesions there is usually a considerable bacterial exudate, a drop sometimes forming at the lower edge of the spot.

A microscopic examination of the earliest visible lesions shows that the bacteria are restricted to a very small area in the center. The water-soaked margin is at first free from bacteria, but, later, they spread into all the surrounding tissues. Progress is most rapid along vascular tissues resulting in the elongated streaks. The infected cells remain turgid for a considerable time if the atmosphere is moist and not too warm, but, eventually, the infected areas collapse and become thin and assume various shades of yellow or brown (Fig. 1, B). The rhizomes show no trace of infection.

ISOLATION

The bacteria taken directly from leaf lesions do not grow vigorously in artificial media. Four to 5, often 8 to 10 days, are required for the production of visible colonies in poured plates. Growth seldom occurs if the temperature is above 28° C., or if the agar surface becomes somewhat dry. By selecting young lesions and providing favorable temperature and humidity conditions, pure cultures of the organism are easily obtained. A peculiarity noted in isolations made directly from the leaf is that only the thickly sown plates developed colonies. It seems that single, widely separated bacteria are unable to multiply in the new and more or less unfavorable environment. If old lesions are used for isolating, the fast-growing secondary organisms, usually or often present, are very likely to develop and occupy the medium before the slow-growing parasite gets started. After several weeks in artificial media, the bacteria become better adapted to the new conditions and more rapid and abundant growth can be obtained.

INOCULATION

To test the pathogenicity of the bacteria isolated from the iris leaf lesions, iris plants were sprayed with a suspension of the bacteria in water. The first test was on outdoor plants without any protection from heat and dryness. Infection was not apparent until more than 3 weeks after inoculation and then only as very small, yellow spots. Six weeks after inoculation, during

a continued rainy period, a considerable amount of infection developed on these plants.

Repeated experiments demonstrated that a moist atmosphere maintained for 5 to 6 days or more after inoculation was the chief external factor necessary for producing good infection and development of large lesions. Most of the tests were with potted plants, which could be kept constantly moist or at least protected from drying by placing them under bell jars, in damp chambers, or covering with glassine bags. Under such conditions the infection often reaches a visible stage in 5 to 8 days, but sometimes 10 days or more elapse before there are definite signs of the disease. Once established in the tissues of the leaf and with the humid conditions continued, the bacteria multiply rapidly and may blight whole leaves in a short time.

Certain plants were inoculated and kept moist for only 2 or 3 days. Small, pale yellow lesions without the water-soaked margins were first observed 4 weeks after inoculation. Other plants not kept in a moist atmosphere following their inoculation, showed numerous small lesions in 6 to 8 weeks. Inoculated leaves often showed no visible sign of infection, but, as long as the leaves remained alive, exposure to moisture for several days would cause the development of typical lesions.

Evidently, infection, or perhaps mere entrance of bacteria to the interior of the leaf, occurs very readily; but, unless conditions are favorable, the bacteria may remain quiescent for considerable periods of time, producing visible symptoms only when suitable conditions arise.

Wounding the leaves by pricking with fine needles or by bruising was found not only unnecessary but often detrimental to infection because, under average conditions, the tissues around even tiny wounds became too dry for bacterial invasion, and, under humid conditions, soft-rot organisms often attacked the injured places.

Injection of the bacteria into iris leaves with a hypodermic needle did not produce any visible symptoms of disease in the few plants inoculated.

All visibly infected leaves may be removed from a plant, but those remaining are very likely to develop infection. When *all* leaves were removed, infection did not appear on those subsequently produced.

Iris flowers were inoculated by spraying with bacteria in water without producing any sign of disease before the flowers faded.

Inoculated rhizomes never became infected.

Infection was secured on all of the bearded irises inoculated (Lent A. Williamson, Crimson King, Miranda, Magnifica, Afterglow, Dalmatica, Mother of Pearl, Edouard Michel, and several unknown varieties).

Several other species (*Iris kaempferi, I. missouriensis, I. sibirica, I tenax* and *I. orientalis*) showed typical infections 10 to 18 days after inoculation. Two varieties of bulbous iris (*I. xiphium* ×) and ixias failed to show infection, while parallel inoculations on bearded iris produced typical lesions. The blackberry lily (*Belamcancha* sp.) became infected.

The numerous, small, isolated lesions usual in the slowly developing infec-

tions suggest stomatal invasion, and stained sections of leaves in early stages of infection show that the bacteria spread from the stomatal chamber, first, horizontally through the intercellular spaces to the opposite surface of the leaf and then, longitudinally up and down the leaf. In these sections the epidermis was intact over rather large areas of infection.

MORPHOLOGY

The bacteria are smaller than the average plant pathogens. When taken directly from leaf lesions, single rods are 0.8 to 1.8 μ long and 0.3 and 0.4 μ wide. From well-grown artificial cultures they are slightly larger, 1.0 to 2.0 μ long and 0.3 to 0.5 μ wide. The bacteria are motile by means of a single polar flagellum, 1½ to 4 times as long as the rod. Capsules are very inconspicuous or lacking. No spores or involution forms have been observed. The bacteria are Gram-negative and are not acid-fast. The writer found these bacteria unusually difficult to stain. All the usual stains and methods were tried, but the slightest washing, even with water, removed much or all of the color.

CULTURAL CHARACTERS

On beef-infusion,[4] peptone-agar plates the colonies are yellow (Mustard Yellow Ridg.),[5] circular, entire, smooth; striated interior markings, sometimes homogeneous, transparent, and viscid. Growth is slow. Colonies are usually less than 1 mm. in diameter in 4 to 6 days after inoculating plates in the usual poured-plate method. Well-separated colonies sometimes reach a diameter of 5 to 6 mm. in 3 weeks. On beef agar slants, growth is only moderate. Clouding is thin in beef broth and growth is mostly at the surface in the form of yellow pellicles and rims. Beef agar plus 0.2 per cent starch is a favorable medium for the organism. In all the beef-infusion cultures, numerous, tiny crystals form and the growth is extremely viscid, even tough and difficult to remove from the agar surface. This viscidity gradually disappears when cultures are 4 to 6 weeks old. In beef-extract agar and broth the growth is even less than in the beef-infusion media and it shows no trace of viscidity.

If the surface of the agar remains moist and the temperature favorable, growth develops as a smooth, thin, continuous layer. Under less favorable conditions, growth develops, if at all, as tiny, isolated colonies.

On potato there is a slight to moderate growth. Milk is completely peptonized in 15 to 20 days. Litmus in milk is not reduced but becomes dark blue. Methylene blue in milk is slowly reduced. In Fermi's and in Uschinsky's solutions growth is very slight. In Cohn's solution there is no growth.

The addition of 1 to 2 per cent of sodium chloride to beef broth greatly reduces growth and 3 per cent prevents growth.

In beef gelatin there is moderate growth but no liquefaction.

In blood serum, very scanty growth and no liquefaction.

[4] Beef infusion was used in all the beef media unless otherwise stated.

[5] Ridgway, R. Color standards and color nomenclature, 53 plates. (Washington.) 1912.

Starch is moderately hydrolyzed.

Nitrate reduction is positive, varying from a weak to a moderate reaction in the several isolates tested.

Ammonia and hydrogen sulphide are produced in small amounts.

All tests for indol were negative, though the bacteria grew well in peptone solution and also in tryptophane solution.

In synthetic media recommended in the Manual of Methods[6] plus various carbohydrates, growth and reactions were so slight or lacking that no conclusions regarding fermentation were warranted. In beef extract plus carbohydrates, growth was scanty to abundant. This medium plus 1 per cent glycerine produced abundant, strong yellow growth; with dextrose the growth was considerably less, only a pale yellow film on the slants with somewhat thicker and deeper yellow in the V. With sucrose and lactose still less growth developed than in the dextrose. At no time was there any indication of acid formation (Brom cresol purple was used as the indicator). Repetitions of these tests gave the same results.

The optimum pH range for growth in beef media is 6.5 to 7.5 (growth is perhaps slightly better at 7.5 than at 7.00). Growth is slight at 5.0 and at 8.0.

The minimum temperature for growth is 5° C. or lower (growth is visible in 8 days at 5° C.). The maximum temperature is 32°; the optimum 26° to 27°; the thermal death point is 44° to 46°. Growth occurred only rarely at 32°; not always at 31°, and even at 30° growth was slow, scanty and erratic. Cultures that failed to grow in 10 to 20 days at 31°, 32° or 33° would sometimes produce growth after removal to room temperature. But no growth occurred after 10 to 14 days at 34° or 35° C.

Beef-agar cultures and also sterile sand and garden soil to which broth cultures were added were stored at −17.8° to −20° C. Transfers made at intervals up to 17 months showed the bacteria alive and vigorous. Iris plants (bearded types) were typically infected with transfers from these long-frozen cultures.

Drops of beef broth cultures were dried on cover glasses at 27° C. Tests showed that vitality was retained for 5 to 6 days. Occasional growth occurred later but none after 10 days' desiccation.

Exposure to direct sunlight kills the organism in 5 minutes or less.

TECHNICAL DESCRIPTION

Bacterium tardicrescens

Short rods, solitary or in short chains; cells 0.8 to 1.8 μ long by 0.3 to 0.4 μ wide, or somewhat larger in well-established cultures. Motile by means of a single polar flagellum, 1½ to 4 times the length of the cell; not conspicuously capsulated. Aerobic; Gram negative; not acid-fast.

On nutrient agar, colonies are slow-growing, circular, flat, smooth, transparent, yellow; beef broth slightly clouded but with moderate yellow pellicle and rim. Gelatin is not liquefied; nitrates are reduced; starch is moderately digested; milk is peptonized; no indol

[6] Society of American Bacteriologists. Committee on bacteriological technique. Manual of methods for pure culture of bacteria (loose leaf). The Society, Geneva, N. Y., 1923 to date.

is formed; ammonia and hydrogen sulphide are formed in moderate amounts. Optimum temperature for growth, 26° to 27° C., maximum 32°, minimum 5° or lower; thermal death point, 44° to 46°. Sensitive to desiccation and to sunlight, but resists freezing for long periods.

The bacteria do not stain easily or well with the usual bacteriological stains.

Pathogenic to *Iris germanica* and various other species and varieties of bearded iris. Also to *I. sibirica, I. cristata, I. missouriensis, I. kaempferi, I. tenax, I. orientalis* and *Belamcanda* sp. (Blackberry lily). Producing leaf lesions of considerable size.

Specimens of diseased iris leaves have been deposited in the mycological collection of the Bureau of Plant Industry.

SUMMARY

A bacterial leaf blight of iris, now known to occur in a number of localities from Alabama to Massachusetts, is described. The most conspicuous symptom of the disease is the occurrence on the leaves of water-soaked areas, mostly as elongated streaks, which later collapse and become either dry or soft rotted, depending on the amount of atmospheric moisture. Rhizomes are not affected. Infection progresses slowly, except in periods of rather moist weather; but the organism survives in the leaf tissues and renews activity whenever favorable conditions arise. Pahtogenncity of the organism isolated from diseased iris was proved by artificial inoculations, which are easily affected by spraying healthy leaves with water containing the parasite. The organism has but slight resistance to desiccation and to sunlight, but it is quite resistant to low temperatures. Clean culture and exposure of the soil to sun and drying conditions and removal of all leaves in late fall or winter would probably aid considerably or prevent infections that most likely arise from bacteria that have overwintered in the soil or in old infected leaves.

Many, perhaps all, of the bearded irises are susceptible to this disease. *Iris cristata* is susceptible, *I. subirica, I. missouriensis, I. kaempferi, I. tenax, I. orientalis and Belamcanda* sp. (Blackberry lily) became infected following artificial inoculation.

Cultural characters and a technical description of the causal organism are given.

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RESISTANCE AND SUSCEPTIBILITY TO CURLY TOP IN VARIETIES OF SQUASH, CUCURBITA MAXIMA[1]

B. F. DANA[2]

(Accepted for publication April 14, 1938)

The curly-top virus has long been recognized (1, 9) as the cause of a very important disease of sugar beets in States west of the Rocky Mountains. The same virus (2, 3, 5, 7, 8, 10) has been found to attack a large number of vegetable crops throughout the same area.

[1] Data presented are based on investigations carried on during the period 1928 to 1937 by the Division of Fruit and Vegetable Crops and Diseases of the Bureau of Plant Industry, United States Department of Agriculture cooperating with the Oregon Experiment Station. Field tests for resistance have been conducted on the Umatilla Field Station, Hermiston, Oregon, where H. K. Dean, Superintendent, has assisted in general over-

Resistance to this virus is of special importance, since control measures for the insect vector, the beet leaf hopper, *Eutettix tenellus*, are lacking. Investigations of resistance in vegetable varieties to the curly-top virus have been carried on in the Pacific Northwest since 1928. Of special interest to vegetable growers and canners are the results obtained in field studies of varieties of squash belonging to *Cucurbita maxima*.

TESTS FOR RESISTANCE TO CURLY TOP

Seed Stocks

Seed of commercial varieties was obtained from seedsmen in the United States and abroad. Tests of this material as it became available were carried on throughout the period from 1928 to 1937. Much additional material was obtained through the Division of Plant Exploration and Introduction of the Bureau of Plant Industry and tested as received in 1930, 1935, and 1937.

Test Plots and Methods

Field tests for resistance have been carried on at the Umatilla Field Station. In the main, the results reported here have been obtained by exposure of test plants to natural infection. The insect vector has migrated into the plots from nearby breeding grounds in sufficient number to introduce and spread the virus generally throughout the plantings. Sugar beets, a favored breeding host for the vector, have been grown in the plots among the test plants to attract this insect and provide conditions favorable for its increase.

The natural abundance of the vector and the extreme susceptibility of the squash varieties to the curly-top virus have resulted in satisfactory elimination of susceptibles among test plants. This has made unnecessary the continued use of the inoculation methods used in early tests. Most of the varieties as indicated in table 1 were included in test plots for more than one year.

VARIETIES SHOWING LACK OF RESISTANCE TO CURLY TOP

Strains Obtained from Seedsmen

Data from tests of varieties for resistance to curly top are presented in table 1, where the percentage of plants affected is entered for all of the strains tested. Infection was uniformly very severe in all but the Marblehead variety. The percentage of crop loss in susceptible varieties closely paralleled that of disease, making it unnecessary to enter separate figures. For most of the varieties data are available for more than one year. The mate-

sight of the test plots and in providing facilities and services of laborers. Published with the approval of the Director of the Oregon Agricultural Experiment Station as Technical Paper No. 287 from the Division of Botany.

[2] Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture. R. F. Wilbur, Agent, conducted test plots in 1928 and 1929. Andrew Steiner, Agent, conducted the test plots in 1930 and assisted in 1931, 1932, 1933 and has assisted intermittently in breeding work and greenhouse work from 1934 to 1937.

rial tested in 1933 to 1937 consisted mainly of varieties not included previously or for which additional data were desired. Several of the varieties tested in 1929 did not show complete infection, but the fact that these varieties showed complete susceptibility in tests of earlier and later years indicates that in 1929 there were susceptible plants of these varieties that escaped infection.

TABLE 1.—*Tests for resistance to curly top in varieties of Cucurbita maxima*

Varieties	Percentage of plants infected for years tested									
	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937
Aizu-Kust								100		
Arikara					100					
Banana	100	75	100	100		100			100	
Boston Marrow	100	100	100	100		100				
Blue Hubbard		100[a]	100				100[e]			
Beau-Dessert					50				100	
Buttercup					100					
Bay State					100[a]					
Delicious		80	100						100	
Estampes					100[a]			100		
California Field								100		
Essex Hybrid		100	100		100					
Golden Hubbard	100	100[b]	100							
Gilmore			100				100			
Golden Delicious					100					
Hubbard			100	100		90			100	
Improved Hubbard	100	95							100	
Kitchenette		100	100							
Orange Marrow			100		100				100	
Mammoth Chili	100	90	100							
Mammoth Whale		50			100					
Mammoth King				100						
Mammoth Melon					100					
Sibley (or Pikes Peak)	100	90	96	100[a]						
Pink Banana							100			
Plymouth Rock								100		
Quality		90					100			
Warted Hubbard		100								
Warren			100		100					
Winnebago			100				100			
Victor			100						100	
Valpariso Red					100					
Hundred Weight					100[b]					
Vermont Hubbard					100					
Zucchetta Nana del Tronco					100					
Umatilla Marblehead	0	20[e]	4	5–7	20	0	0			
Yakima Marblehead						10	0			10[d]

[a] Two strains.
[b] Three strains.
[c] Eight strains.
[d] Crop loss, 0–5%.
[e] Crop loss, 5–10%.
(Note: Percentage of crop loss was total in most cases, but very closely paralleled the percentage of disease.)

Plant Introduction Stocks

Foreign strains of squash were included in test plots for single years only, due to limited supplies of seed. In some cases, seed germination was poor, so that very few plants were obtained. For these reasons, data on resistance of foreign strains are not reliable for every strain handled. However, the data are considered to be more truly representative of all the strains, since, with 2 exceptions, all showed complete susceptibility. Most of the affected plants died before fruit was produced. Almost no acceptable fruits were produced on any of the diseased plants. Total crop loss is entered for all but 2 of the strains, although some cull fruit was produced.

Since the one strain in 1937, which showed 20 per cent of infection and crop loss, was variable as to plant character, further tests will be necessary to evaluate satisfactorily resistance in this strain. The strain showing 75 per cent of infection and crop loss in 1931 has little promise because of the high percentage of disease (Table 2).

TABLE 2.—*Percentage incidence of curly top and consequent crop loss observed in test plots of foreign strains of squash grown in the field at Hermiston, Oregon*

Year	Strains tested	Number of strains showing stated percentage of disease	Percentage crop loss
1931	2	1 100 1 75	100 75
1935	11	11 100	100
1937	27	26 100 1 20	100 20

VARIETY SHOWING RESISTANCE TO CURLY TOP

The Marblehead Strains

Data for the Marblehead strains (Table 1) show very low percentage of injury compared with other varieties of this species. The figures given are for the stocks obtained from growers or seedsmen. These strains are very heterozygous for such characters as color and shape of fruit and of seed. The presence in every commercial field of an occasional plant seriously injured by curly top, while the rest of the plants are disease-free or relatively uninjured, indicates that these strains are heterozygous also for resistance to the curly-top virus.

Inbred lines, not listed in table 1, have shown varying degrees of resistance. Some have been very resistant, others have been severely injured. Those strains that have shown strong resistance have not been quite so generally high in quality as the original heterozygous strains. The isolation of additional inbred strains may be necessary to obtain a combination of the highest quality with resistance. The available inbred lines are being used in a breeding program for the production of different types of resistant squashes. They also are being used to determine the nature and behavior of resistance to the curly-top virus.

The origin of these Marblehead strains is somewhat of a mystery. The Yakima strain has been grown in the Yakima Valley of Washington for 25 to 30 years. The Umatilla strain has been grown on the Umatilla Station in Umatilla County, Oregon, for about the same length of time. The 2 strains, according to local observers, have been kept separate for this length of time. The similarity of the 2 strains, however, would suggest a common origin.

Both strains have characters distinct from all members of the Hubbard group with which the original Marblehead, introduced by Gregory, was grouped by Castetter and Erwin (4) and Erwin and Haber (6). From the standpoint of character and resistance these strains of Marblehead should be placed in a separate group.

Shape of fruit ranges from round through cylindrical-oval, elongated-oval, top-shape, egg-shape and various tapered or pointed forms. Color is variable as to ground color, striping, spotting, netting, and combinations. Of these, dark green and slate predominate as the ground color, with a small percentage of orange. Over the ground color may be spread a fine slate netting or a regular or irregular pattern of slate stripes and blotches. Slate-color fruits predominate; a very few are partially or completely orange-color.

Seed color in different fruits ranges from ivory to tan. Shape and size of seed and thickness of seed coat also are variable. Quality in these strains is good to excellent. The original Marblehead strain appears to be extinct and physical comparison with these Northwest strains for more accurate determination of relationship has not been possible.

It is indeed singular that one variety should show strong resistance, while other varieties of this species show entire lack of resistance. These strains of Marblehead present no physical characters that are not typical of the species, *Cucurbita maxima*. It is very interesting also that satisfactory quality should be combined with such strong resistance and vigor.

SYMPTOMS OF CURLY TOP ON SQUASH VARIETIES

Symptoms on Susceptible Varieties

In the seedling stage, squash plants usually succumb quickly to curly-top infection. Under high temperatures the incubation period of the virus is shortened. Exposure to drying winds, hot sun and low soil moisture greatly increases injury from the disease in plants at any stage of development.

If the young seedling is inoculated by virus-carrying leaf hoppers as soon as it emerges above the soil, it may die before true leaves appear. Death of the plant follows infection less suddenly as the plant grows older. Seedlings or young plants that die quickly do not show characteristics distinctive for this disease alone. Growth is stopped and the plant gradually withers and dies (Fig. 1, A).

Older plants may be severely injured or killed, especially if growing conditions are unfavorable. The mature leaves are not changed in form but

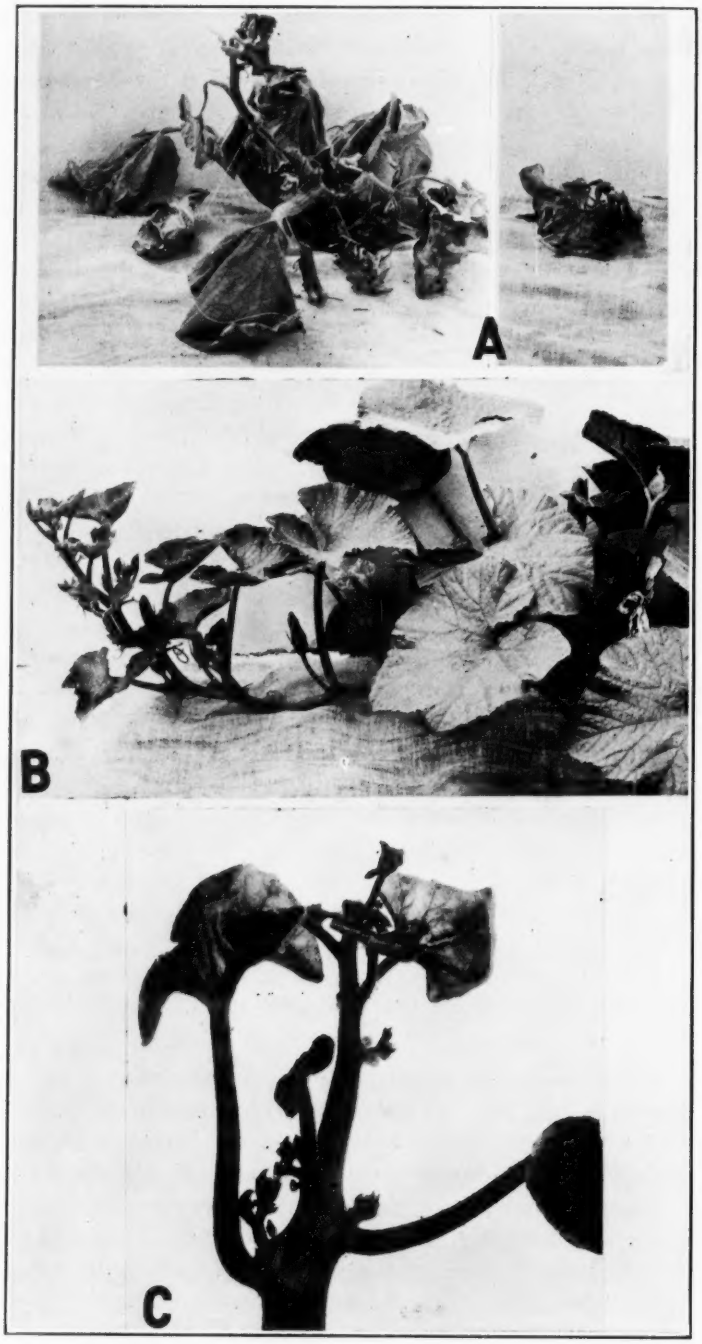

FIG. 1. A. Young plants of Boston Marrow squash, *Cucurbita maxima*, dying from curly-top infection. B. and C. Curly-top infections on tips of runners: B, Sibley squash, *C. maxima*; C, Marblehead, *C. maxima*.

may gradually yellow and die. New growth is definitely dwarfed; internodes are shortened; and leaf blades may develop a savoyed surface or roll upward at the margin. Bending upward of the tip of the runner is very characteristic (Fig. 1, B). Blossoms on these diseased shoots usually do not set fruit. New growth on diseased plants may appear darker green than the older, deteriorating foliage.

Wilting is not characteristic of curly-top infection in squash plants. Instead, there may be gradual deterioration ending in death. Distinctive color differences by which a diseased plant may be identified are lacking. Infection late in the life of the plant reduces size of fruit and number of fruits that may mature.

Symptoms of Curly Top on Resistant Marblehead

The character of new growth at the tips of runners may be the only indication that a plant is diseased. Shortened nodes and side shoots give rise to clustered growth at the tips of runners. Dominance of the growing tip is lost and a witches' broom type of growth is formed. The individual branches often become thicker than the runner from which the abnormal structure arose.

Foliage is dwarfed and of lighter color than normal leaves. Leaf blades on the younger leaves roll upward and are somewhat more rigid than normal leaves (Fig. 1, C). Both abortive and abnormal flower development has been found on these abnormal runner-tips or "witches' brooms."

During the fall of 1937 several cases were found of partial transformation of petals and pistil of pistillate flowers to leaf-like structures. In one case the development of phyllody in the female flower was striking, with still more complete transformation of petals, style, and stigma into leafy structures. Phyllody was found only on the strongly developed "witches'-brooms" and appeared to be correlated with increased branching as a vegetative response to the curly-top virus. Similar vegetative stimulation by the virus, either in the form of witches' brooms or phyllody, has not been seen in susceptible varieties of the species, *Cucurbita maxima.*

SUMMARY

Data are presented showing that horticultural varieties of the species, *Cucurbita maxima,* exhibit extremes of both susceptibility and resistance to the curly-top virus without intermediates between these extremes.

Two strains, known as Umatilla Marblehead and Yakima Marblehead, are outstanding for resistance to the curly-top virus. Other tested varieties of this species show extreme susceptibility. The two resistant strains appear to have been developed in the Pacific Northwest. Their similarity suggests a common origin, although local observers insist that they have been grown separately for 25 to 30 years. They are extremely variable and yet nearly identical in their variations as to shape and color of fruit and seed. They

are also heterozygous for resistance, since a few plants in either strain show severe injury from curly top.

Symptoms of curly top on susceptible and on resistant varieties are described.

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DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES,
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THE PRODUCTION OF A NEW PHYSIOLOGIC RACE OF SPHACELOTHECA SORGHI[1]

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(Accepted for publication April 21, 1938)

By crossing monosporidial lines of *Sphacelotheca sorghi* (Lk.) Cl. the writer has obtained a physiologic race that differs from those hitherto described. Although the new race has not been found in nature, it merits attention as evidence that new parasitic races may arise as a result of crossing between haploid biotypes within a species.

The new race was produced in the following way: In 1929 Dr. L. J. Tyler obtained a collection of *Sphacelotheca sorghi* from Amarillo, Texas, and isolated individual sporidia from promycelia of several chlamydospores. The resulting monosporidial lines were inoculated into sorghum in various sexually compatible combinations, and smutted heads resulted. Dr. Tyler kindly gave the writer some of his material, and further experiments were made. The history of the crosses resulting in the production of the new race is summarized below and illustrated diagrammatically in figure 1.

[1] Paper No. 1604 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

Crosses		
Numerical designation	*Gametic parents*	*Color of smutted heads*
122	Tex. $B2_I \times$ Tex. $C2_3$	Gray
123	122 B4 $\times$ Tex. $A3_I$	Gray
124	123 A3 $\times$ 123 A4	Gray, brown, and intermediate

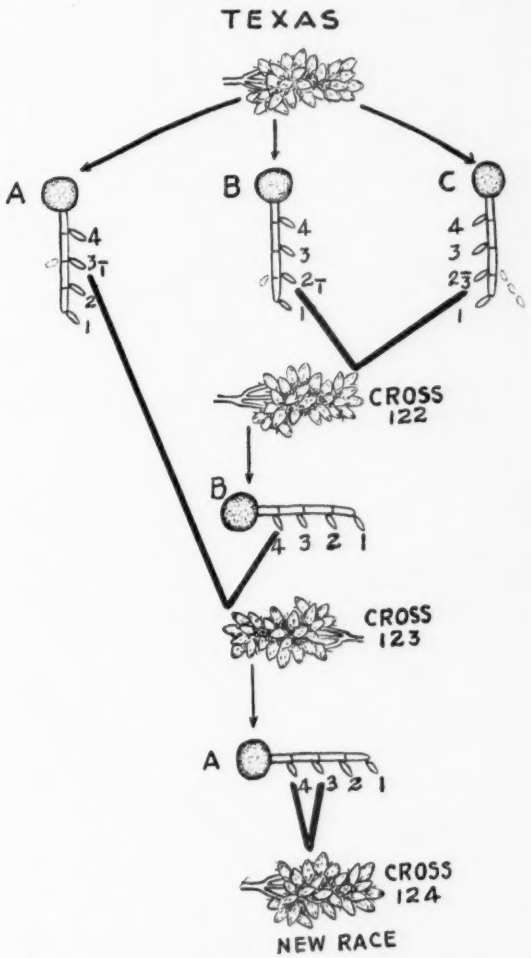

FIG. 1. Diagrammatic representation of the origin of a new race of *Sphacelotheca sorghi*.

The following explanation may make the procedure clear. The chlamydospores designated Tex. A, Tex. B, and Tex. C were obtained from a single smutted kernel of the smut collection sent Dr. Tyler from Amarillo, Texas. The monosporidial line derived from sporidium Tex. $B2_I$ was crossed with that derived from Tex. $C2_3$. This was done by growing the lines separately in potato-dextrose broth, then mixing the two liquid cultures and injecting the mixture into Minnesota Amber sorghum plants by means of a hypodermic syringe. The resulting chlamydospores were designated cross 122.[2] Sub-

[2] The system of designation is shown in figure 1, but the significance of the sub-numbers may not be clear. When several sporidia are abstricted or taken from the same cell

TABLE 1.—*Percentage of smutted heads in sorghum inoculated with 5 physiologic races and "Cross 124" of Sphacelotheca sorghi*

Variety, selection, or hybrid	Accession number	Race and percentage of smutted heads[a]										
		1 (Kafir)		2 (Milo)		3 (Feterita)		4 (Feterita)		5 (Feterita)		"Cross 124" (Hegari-Kafir)
		M[a]	V[a]	M	V	M	V	M	V	M	V	V
Dwarf Yellow milo	K.B. 2515	0	0	18.0	24.0	0	1.0	0	0	0	0	0
Dwarf Yellow milo	C.I. 332	0	0	13.2	17.1	0	0	0	0	0	0	0
White Yolo	K.B. 2525	1.2	0.8	35.3	31.8	0	0	12.6	10.7	0	0	0
Pierce Kaferita	K.B. 2547	0	0	0	0	20.6	[b]	0	1.8	0	[b]	0
Feterita × Kafir	F.C.I. 8917	0	0	0	0	2.4	8.4	0	0	0	[b]	1.1
Feterita	S.P.I. 51989	0	0	0	0	2.6	0.5	0	0	0	0	0
Kafir × feterita	H.C. 2423	0	0	0	0	18.4	10.6	0	0	41.6	[b]	0
Hegari	K.B. 2518	0	0	13.6	–	0	–	–	–	–	–	6.1[c]
Reed Kafir		23.1	–	16.5	–	23.0	–	–	–	–	–	85.3[c]
Schrock		0	–	9.2	–	43.5	–	–	–	–	–	62.0[c]

[a] In column M are given the figures obtained by Melchers, Ficke, and Johnston at Kansas in 1929, while in column V are given the average percentage of smutted heads in triplicate rod rows in the writer's tests at University Farm, St. Paul, Minnesota, in 1936.

[b] Data inconclusive, but do not affect the results.

[c] Average of 2 years' results (1935 and 1936). Symbol – indicates no test.

sequent procedures were similar until cross 124 was obtained. This proved different in pathogenicity from any race hitherto described.

Forty-eight varieties of sorghum[3] were inoculated with chlamydospores from a gray head of cross 124, in the field at University Farm, St. Paul, Minnesota, in the summer of 1935.[4] As the results of these tests indicated that Cross 124 was different from the 5 races of *Sphacelotheca sorghi* previously recognized as a result of the investigations of Tisdale, Melchers, and Clemmer[5] and Melchers, Ficke, and Johnson,[6,7] further tests were made in 1936. For comparison, inoculations were made with the 5 races also, the chlamydospore material having been obtained through the kindness of Professor L. E. Melchers (Table 1).

From table 1 it is clear that Cross 124 differs from Race 5 because Kafir × feterita (H. C. 2423) is highly resistant to 124 and susceptible to Race 5. It differs from Race 4 because White Yolo (K. B. 2525) is highly resistant, while it is susceptible to Race 4. It differs from Race 3 because Pierce Kaferita (K. B. 2547), feterita (S. P. I. 51989), and Kafir × feterita (H. C. 2423) are highly resistant, whereas they are attacked by Race 3. It differs from Race 2 because the Dwarf Yellow milos and White Yolo are highly resistant, while they are susceptible to Race 2. Cross 124 differs from Race 1 because 124 attacks Hegari, to some extent. Cross 124 also differs from Races 1, 2, and 3 in its effect on other varieties, such as Schrock and Reed Kafir, on which it produced 62.0 and 85.5 per cent of smut, respectively. Cross 124 differs from Races 1 and 4 in the color of the peridium, which is gray to brownish, like that of Races 2, 3. and 5, while with Races 1 and 4 the color of the peridium is brown.

It appears, therefore, that a new race of *Sphacelotheca sorghi*, which can be designated Race 6 (Hegari-Kafir race), has been developed by crossing monosporidial lines derived from the promycelia of chlamydospores obtained from a single smutted kernel.

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of a promycelium, the first one is given the serial number of the appropriate cell, counting from the tip cell, and each subsequent one is designated by a sub-number. Thus, Tex. $C2_5$ means the fourth sporidium formed on cell 2 of the promycelium produced by chlamydospore C of the Texas smut collection.

[3] The writer is greatly indebted to Professor L. E. Melchers, Professor J. H. Parker, and Dr. J. H. Martin for furnishing seed.

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PHYTOPATHOLOGICAL NOTES

A Race of Ustilago avenae Capable of Infecting Black Mesdag Oats.[1]—The variety Black Mesdag has long been used as a smut-resistant parent in oat-breeding programs. Reed,[2] in 1932, and Stanton, *et al.*,[3] in 1934, have shown that certain races of covered smut, which they designate as *Ustilago levis*-Fulghum, can attack this variety. Until recently, Black Mesdag always has been resistant to loose smut when grown at University Farm, St. Paul Minnesota. In 1936, however, 2 collections of loose smut (*Ustilago avenae* (Pers.) Jens.) from Phillipsburg, Kansas, and Bartlesville, Oklahoma, produced 23 and 30 per cent of smutted heads, respectively, on this variety. In 1936 each collection was increased on Black Mesdag and on Anthony, a generally susceptible variety.

In 1937, 7 varieties of oats were inoculated with these collections and with 2 others that had been increased on Anthony and Gopher (Table 1). Chlamydospores of the Kansas and Oklahoma collections that had been produced on Black Mesdag in 1936 caused a far higher percentage of infection in this variety than those of the same collections that had been produced on Anthony. Material of the Oklahoma collection taken from Black Mesdag was much more pathogenic also to Double Cross II 20–220, (Minota × White Russian) × Black

TABLE 1.—*Pathogenicity of four collections of Ustilago avenae after having been increased on different varieties*

Source of inoculum		Variety inoculated in 1937 and percentage of infection[a]						
Original collection	Variety on which produced in 1936	Anthony	Gopher	Double Cross	Black Mesdag	Bond	Markton	South Dakota No. 165
Oklahoma	Anthony	90	43	3	15	0	1	0
	Black Mesdag	87	49	76	84	1	3	0
Kansas	Anthony	31	0	28	26	0	0	0
	Black Mesdag	43	0	26	48	0	0	0
Nebraska	Anthony	49	4	0	1	0	0	0
	Gopher	44	6	0	0	1	0	0
Indiana	Anthony	89	67	0	0	1	1	4
	Gopher	95	88	0	0	3	5	0

[a] Percentage of infection is based on the number of smutted heads in replicated eight-foot rows at University Farm, St. Paul, Minnesota, in 1937.

[1] Paper No. 1602 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Reed, G. M. Reports on research for 1932. Plant Pathology. Brooklyn Bot. Gard. Rec. **21**: 42–46. 1933.

[3] Stanton, T. R., G. M. Reed, and F. A. Coffman. Inheritance of resistance to loose smut and covered smut in some oat hybrids. Jour. Agr. Res. [U. S.] **48**: 1073–1083. 1934.

Mesdag, a hitherto resistant selection, than that from Anthony. This, however, was not true of the Kansas collection. Bond, Markton, and South Dakota No. 165 remained resistant to these collections regardless of the variety on which the smut had been increased.

Two other collections, from Indiana and Nebraska, were not appreciably different in pathogenicity when increased on varieties (Anthony and Gopher) susceptible to smut.

The results confirm the observations of Dillon-Weston,[4] Nicolaisen,[5] and others that the virulence of a smut collection for a resistant variety may be increased by successive inoculations of that variety, owing to the development of an increased proportion of the biotypes capable of developing on it. It is clear that a collection of smut may comprise many biotypes differing in pathogenicity for different varieties. Naturally, only those biotypes that are capable of attacking a given variety will increase on it; consequently there is a screening effect. If even a few heads become normally smutted when a resistant variety is inoculated with a collection, the variety must be susceptible to one or more biotypes in the collection, unless, of course, the variety itself is not pure; and when the variety is inoculated with its own smut the percentage of infection is likely to increase. Obviously, there are biotypes of *Ustilago avenae* that can attack Black Mesdag, which has been used extensively as a smut resistant parent in breeding work.—EDWARD K. VAUGHAN, formerly of the Minnesota Agricultural Experiment Station, now in Division of Fruit and Vegetable Crops and Diseases, U. S. Department of Agriculture, Washington, D. C.

A Seed-borne Disease of Sweetclover.[1]—In the course of the examination of the life histories of some fungi on sweetclover, *Melilotus* spp., it has been found that two species of *Ascochyta* with the same spore measurements, but with different life histories and very different pathological consequences, occur on this forage crop. The more common and well-known species is *Ascochyta lethalis* Ell. and Bart., more properly designated *Mycosphaerella lethalis* Stone from the name of its ascigerous stage. The less common species to which this note calls attention is *Ascochyta caulicola* Laubert for which no ascigerous stage has been found. While these two fungi are not readily distinguished by usual mycological procedure—the latter name has often been regarded as a synonym of the former—they are easily distinguished in culture and by the pathological conditions they effect.

The disease caused by *Ascochyta lethalis* has been called black stem by Johnson and Valleau,[2] a most appropriate name were it not for the fact that other fungi cause blackening of stems. This fungus usually fruits sparsely

[4] Dillon-Weston, W. A. R. Resistance of wheat varieties to bunt. Nature **123**: 243. 1929.

[5] Nicolaisen, W. Die Grundlagen der Immunitätszüchtung gegen *Ustilago avenae* (Pers.) Jens. Zeitschr. f. Pflanzenzüchtg. **19**: 1–152. 1934.

[1] Contribution from the Division of Forage Crops and Diseases in cooperation with the Wisconsin Agricultural Experiment Station.

[2] Johnson, E. M., and W. D. Valleau. Black-stem of alfalfa, red clover and sweet clover. Ky. Agr. Expt. Sta. Bull. 339. 1933.

and tardily on blackened areas. In strong contrast with this, the caulicolous *Ascochyta* causes bleached areas, sometimes accentuated by a brown border, and in vigorously growing plants it causes a bending of stems, which has suggested the name "gooseneck" for the disease. The fungus fruits abundantly on the lesions as soon as they appear. The common black stem fungus, likewise, occasionally produces grayish white lesions with brown borders,[3] but these can usually be distinguished by the less abundant and less conspicuous pycnidia upon them. Often both fungi occur on the same plant.

The disease caused by *Ascochyta caulicola* was described in Germany in 1903.[4] Apparently it has not been recorded in this country. Collections of what appears to be this disease are, however, in the writer's herbarium from the following sources:

Huntley, Montana. Collected Aug., 1921, by C. S. Scofield.

Urbana, Illinois. Collected June, 1931, by F. R. Jones.

Holgate, Ohio. Collected June, 1932, by F. R. Jones.

Elks County, Pennsylvania. Collected June, 1934, by George Zundell.

Lincoln, Nebraska. Collected June, 1937, by E. A. Hollowell.

The disease has been seen this summer in Kansas, Nebraska, Iowa, Illinois, and Wisconsin. In Wisconsin it has been found chiefly in pastures, where it is assumed that it must have been brought by seed, as it has not been found on roadside plants in this State except in one location. It has been found in occasional plants in seed fields in Nebraska and Illinois, but nothing is known of its frequency in such fields.

In young, vigorous, infected plants the fungus has been isolated from all parts except the root, even though lesions were found only on a portion of the stem. The fungus has been isolated also from seed from diseased plants, and in one instance plants from such seed appeared healthy when kept all winter in the greenhouse, but became covered with lesions about two weeks after they were placed out of doors in frequent rains. Thus the fungus appears to be systemic to some degree. Because of these characters, the disease deserves attention, especially in seed sources.—FRED REUEL JONES, Madison, Wisconsin.

REPORT OF THE 1938 ANNUAL MEETING OF THE SOUTHERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 1938 annual meeting of the Southern Division of The American Phytopathological Society was held in connection with the meeting of the Association of Southern Agricultural Workers on February 2–4, inclusive, in Atlanta, Georgia. The morning of February 2, was devoted to a general session, when papers on a variety of plant-disease problems were given. The afternoon of February 2, all of February 3, and the forenoon of February 4 were devoted to the presentation of papers and general discussions dealing with various of the cotton-disease problems, particularly, seedling diseases, Fusarium wilt, and Phymatotrichum root rot. This part of the program was under the supervision of the Cotton Disease Council, which was considered as constituting a part of the Southern Division. Approximately 75 plant pathologists and visitors attended the general session

[3] See footnote 2.

[4] Laubert, R. *Ascochyta caulicola*, ein neuer krankheitserreger des steinklees. Arb. Biol. Reichsanstalt f. Land- u. Forstwirtschaft 3: 441–443. Illus. 1903.

on the morning of February 2, and approximately 50 attended the subsequent sessions of the Cotton Disease Council.

A root knot conference was held in the afternoon of February 4. Dr. G. Steiner of the U. S. Department of Agriculture, was the principal speaker at this conference. The remainder of the program was devoted to informal discussions of root knot problems.

An informal meeting of several members of the Tobacco Disease Council was held on the evening of February 3. The discussion was devoted primarily to policies and functions of the Council.

A short business meeting was held on the afternoon of February 2. The following officers were elected: President, G. M. Armstrong; Vice-President, V. H. Young; Councilor, A. N. Brooks; Secretary-Treasurer, Luther Shaw. A constitution for the Southern Division of the American Phytopathological Society was read and adopted.

The following resolution was adopted by the Society:

Whereas:

The Southern Division of The American Phytopathological Society has lost a friend and esteemed coworker in the passing of Dr. J. J. Taubenhaus.

Therefore be it resolved:

That the Southern Division of The American Phytopathological Society express its sympathy to the bereaved family; and, that the Secretary be instructed to forward a copy of this resolution to the bereaved family, and also to add it to the minutes of this meeting.

Signed:

V. H. YOUNG,
W. D. MOORE,
R. F. POOLE,
(Resolutions Committee)

Titles and abstracts of papers presented at the meeting follow.

LUTHER SHAW,
Secretary-Treasurer.

Eye Spot of Napier Grass. R. K. VOORHEES. Eye spot of Napier grass, *Pennisetum purpureum,* which, in the spring of 1937, was definitely determined to be caused by *Helminthosporium ocellum,* was first observed in the Agronomy fertility plots of the Department of Agronomy, University of Florida, at Gainesville. This organism had been reported previously as causing an eye spot and a ring spot of sugar cane in Cuba, and Florida. No typical ring spots were observed on the Napier grass at Gainesville, although sugar cane affected with ring spot was growing in the immediate vicinity.

The characteristic symptoms of this disease on the leaves become manifest in the appearance of reddish, somewhat oval to elongate spots, measuring 1.5–3 mm. wide by 2.5 mm. long. In cases of heavy infection the leaves wither and die prematurely, the basal leaves dying first and dropping to the ground. It is not uncommon to find the leaf sheaths and stems also heavily infected the individual spots being smaller than those on the leaves but tending to coalesce and form large, brown, sunken areas.

The disease was produced on young plants growing in the greenhouse by inoculating with a water suspension of spores from pure cultures of the fungus. Characteristic spots appeared on the leaves of the plants from susceptible stock, while no spots developed on the plants from resistant stock or the noninoculated checks.

Leaf Blights of Fig in Louisiana. E. C. TIMS and P. J. MILLS. There are two distinct leaf blights of fig in Louisiana caused by *Rhizoctonia* (or *Corticium*). Thread blight (*Corticium stevensii* (Burt)) has been prevalent for many years. Recently a Rhizoctonia blight was found that is rather different from typical thread blight. The fungus mycelium grows up the leaf petioles in a very thin layer, often causing deep cankers, then spreads fan-like on to the leaves. The leaf tissues turn brown and often fall out, producing a shot-hole effect. The leaves quickly shrivel and fall off if conditions are favorable, seldom adhering to the twigs as in the typical thread blight. The Rhizoctonia blight produces no powdery gray to brown mycelial mats on the lower surface of the leaf—basidial stage—and no typical threads or sclerotia, as in thread blight. The two blights have not been observed on the same tree. A rapid-growing *Rhizoctonia,* similar to the forms found widely distributed in the soil, was readily isolated from diseased fig leaves and its pathogenicity demonstrated. This fungus differs in appearance, growth rate, etc., in culture from the rather slow-growing form associated with thread blight. Neither of the organisms has produced the basidial (*Corticium*) stage in culture under our conditions.

Problems in the Germination of Cottonseed. D. M. SIMPSON and G. M. STONE. Seedling stands of cotton are affected by the sensitivity of the planting-seed to adverse condi-

tions. Sensitive seed lots quite often germinate well in the laboratory when tested by the standard method of cottonseed germination, but fail to produce good stands when planted in the field. A new laboratory method of detecting the degree of sensitivity has been tested at Knoxville, Tennessee, and the results show close correlation with field behavior. Essentially, the method is as follows: The seeds are delinted with sulphuric acid, placed in water and vacuumized for five minutes at a negative pressure of 27 inches of mercury, then removed, drained of surplus water, and placed on moist paper towelling for germination at the usual 20–30° C. alternate temperature as in the standard method for cottonseed germination. The weak seeds fail to germinate after being subjected to the vacuum treatment. Studies indicate that sensitivity may be closely associated with the texture or structure of the seed coat.

Lightning Injury to Cotton. A. L. SMITH. Lightning-struck spots in cotton fields vary considerably in appearance. Perhaps the commonest type of injury is the killing of all vegetation in roughly circular areas with marginal intermingling of living, dead and injured plants. Quite commonly, however, little or no sudden killing occurs and exact centers of struck spots cannot be determined. This latter type of lightning injury is particularly confusing to growers, since noticeable symptoms do not appear until sometime later when scattered plants commence wilting and dying. Areas 300 ft. in diameter thus affected have been observed. Presumably, the magnitude of the discharge, soil moisture, and soil type largely determine the extent of injury at the center and the distance of lateral spread.

Plant symptoms are similar in all cases. Plants not killed outright show injury at or near the soil line, which consists essentially of a girdling of the stem. Presumably, the cambium and phloem tissues are killed in a narrow band. Subsequent developments are root starvation, a collar-like enlargement above the injury with adventitious root development, reddening of the foliage and subsequent wilting and dying of the plant.

Comparative Injury of Root-knot Nematodes to Different Varieties and Species of Cotton in Control Experiments Under Irrigation. C. J. KING. Upland varieties of cotton show little injury in soils having a moisture equivalent above 18. At 16 or less the stands often are reduced, and mature plants may appear unhealthy. Egyptian, American Egyptian, Peruvian, and Sea Island varieties are more susceptible. The F hybrids of upland and American Egyptian varieties are intermediate in susceptibility. The ratio of yields for Acala and Pima on adjoining strips of root-knot-free land has averaged 1.8 over a period of several years. Similar tests on infested soils gave a ratio of 3.25. Frequent irrigations during seedling development reduce injuries to Pima cotton, especially when ammonium sulphate is added to the water 1 part in 10,000. Heavy applications of ammonium compounds, calcium cyanamid, and organic fertilizers have been beneficial in reducing nematode injuries. The effects on highly calcareous soils are not lasting. Under Arizona conditions clean fallowing for 3 to 4 years, combined with frequent deep tillage in summer, nearly eradicates nematodes. Reinfection of the treated areas is, however, rapid unless precautions are taken. Rotation of cotton and alfalfa, with each crop maintained for 2 or 3 years is a practical method of control in some areas.

Further Studies of Crinkle Leaf, a Disorder of Cotton Plants Prevalent in Lintonia and Olivier Silt-loam Soils in Louisiana. D. C. NEAL and H. C. LOVETT. The disease of cotton known as crinkle leaf, prevalent in certain Lintonia and Olivier silt loam soils in Louisiana, has been found associated with high acidity, calcium deficiency, and manganese toxicity. This disease results in the formation of puckered, mottled, partially chlorotic, and variously distorted leaves in the early stages; fasciation of branches; and the development of distorted floral buds, flowers, and bolls. Soil from areas in Louisiana affected with crinkle leaf has a consistently lower pH and contains significantly larger amounts of manganese than soil from healthy areas. The disease was produced in cotton plants in sand cultures by the addition of increasing amounts of manganese sulphate. It is readily corrected by calcium and other basic carbonates.

Results of Seed-treatment Tests with Cotton in 1937. S. G. LEHMAN. An attempt was made to determine the relative effectiveness of Ceresan and New Improved Ceresan as seed-treatment materials for cotton. These commercial preparations were applied in such quantities as to add to the seed equivalent units of the active ingredient in the preparations. Two plantings—medium early and medium late—were made.

New Improved Ceresan increased seedling emergence significantly more than Ceresan in the medium-early plantings on Cecil sandy-loam soil at Raleigh and on Norfolk sandy loam near Rocky Mount when the amount of material applied was sufficient to give adequate coverage. In the medium late planting these 2 preparations gave approximately

equal increase of emergence, the increases being barely significant at Raleigh and decidedly significant at Rocky Mount.

Both materials increased the yields by an approximately equal percentage. These increases were generally significantly large when sufficient material had been added to give good coverage of seed except in the medium late planting at Rocky Mount where the increases barely approached significance.

Zinc oxide, copper oxychloride "A," and cuprocide gave significantly large increases in emergence at Raleigh and Rocky Mount in the early planting. In the medium late planting these materials gave small and generally insignificant increases at Raleigh, but larger and significant increases at Rocky Mount.

Ceresan greatly reduced the percentage of sore shin and cotyledonary lesions of angular leaf spot on seedlings. This effect on angular leaf spot was still evident on plants in the late summer.

Results from Treating Cottonseed with 2 Per Cent Ceresan. U. R. GORE. Cotton-seed-treatment tests were conducted by the Georgia Experiment Station in 1937 at 3 locations in south Georgia and one in the Piedmont. In these tests a small factorial design with 2 varieties, nontreated and treated with 2 per cent Ceresan was used. Dixie 14–5 and Coker Clevewilt 6 both germinating 95 per cent were the varieties used in south Georgia. Dixie 14–5 germinating 95 per cent and seed of another variety germinating 30 to 38 per cent were used in the test in the Piedmont. Tests were drill planted at Readsville on March 31, at Hawkinsville and Cordele, April 2. The weather was cool and generally unfavorable for cotton during April. The test at Experiment was a medium late drill planting, May 6. Weather conditions were ideal for cotton.

Results reported here are from data on one year's tests only. Data were secured on (1) seedling stands before chopping, (2) plants per acre at time of harvest, and (3) yield of seed cotton per acre. Analysis of variance of the data shows no significant increases in stand before thinning for Ceresan treated seed at any location. A significant increase in number of plants per acre at harvest was obtained from Ceresan at one location and a significant decrease was obtained at one location. Increases in yield of seed cotton from Ceresan were obtained in 5 out of 8 comparisons. The differences in yield were not statistically significant for Ceresan treatment at any location.

Cottonseed Treatment for Stand Improvement. D. C. NEAL. In cottonseed-treatment experiments conducted in 1937 at Baton Rouge and St. Joseph, La., for the improvement of stands, it was found that Cerasan at the rate of 3 ounces per bushel and Improved Ceresan at a rate of 1 ounce per bushel were about equally effective. Both of these dusts at the above rates were superior to copper oxide.

Delinting cottonseed, either mechanically or by means of sulphuric acid, gave no improvement in stand during the season of 1937 at Baton Rouge, but mechanical delinting did result in a higher yield of seed cotton. Sulphuric acid delinting, on the other hand, gave a significant reduction in both stand and yield in these tests and does not appear promising for Louisiana conditions.

In districts where growers prefer the hill-drop system of planting, mechanical delinting and subsequent dusting with Ceresan or Improved Ceresan at rates of 3 and 1 ounce per bushel, respectively, will likely result in more uniform stands and better yields.

Cottonseed Treatment Gives Larger Yield. W. C. NETTLES. A $1,500,000 increase in the State's cotton crop by seed treatment for only 250,000 acres points the way to still greater results in 1938 and the future.

One acre out of every 6, or 250,000 acres, planted to cotton in South Carolina in 1937 was planted in seed treated with mercury dust known under the trade name of Ceresan. Results indicate more plants and healthier ones, and increased yield. Twenty-four per cent more plants were present on treated rows than on nontreated rows at chopping time.

The most striking result obtained was the reduction in sore shin. Over 60 per cent of the plants grown from nontreated seed had sore shin, while only 16 per cent of those grown from treated seed were so affected, and then very slightly.

On 115 farms scattered throughout South Carolina, treated seed produced 16.7 per cent more bolls than nontreated, which means an extra 205 pounds of seed cotton per acre.

While these figures are based on partial returns, it appears certain that complete returns will strengthen the results, and that the seed treatment on the 250,000 acres alone will increase the value of the 1937 cotton crop by $1,537,500.

The Reaction of Cotton Leaves to Hypodermic Injections of Fusarium vasinfectum. A. L. SMITH. Preliminary studies of methods for differentiating cotton plants for reaction to *Fusarium vasinfectum* indicated possibilities of producing leaf symptoms by hypo-

dermic injections in stems near the terminal bud or in leaf petioles. Symptoms produced were similar to those on naturally infected leaves except that small portions of leaf blades generally were involved. Limited areas becoming browned were bounded by discolored veins that, when cultured, yielded the organism previously introduced. Further inoculations were made in the greenhouse, using 6 varieties varying widely in known reaction. The percentage of inoculated leaves showing wilt symptoms for Clevewilt (resistant) and Half and Half (susceptible) was 88.9 and 83.3 per cent, respectively. Other varieties showed lower percentages of infection. Since these two varieties represent the extremes in known reaction of the varieties studied, it was concluded that no relation exists between natural field infection and the appearance of leaf symptoms following hypodermic injection in leaf petioles. A more extensive study under field conditions, using the same varieties, confirmed the above results.

Technique of Artificial Inocu'ation with Fusarium vasinfectum. D. C. NEAL. In additional studies of wilt-infection technique with cotton seedlings in the greenhouse, results have been secured with 4 varieties of cotton, namely, Delfos 2323, Clevewilt, Dixie Triumph 12, and Half and Half, which appear significant for resistance and conform in some degree to the field behavior of the varieties. Similar trends of resistance were secured when the plants were grown in 2 soil types after being inoculated with 5 different isolates of the fungus grown simultaneously in Czapek's solution. Cultures of the fungus grown on a 4:1 mixture of oats and wheat and subsequently mixed with sand to give a mixture of 5 per cent of the fungus inoculum have been found to be satisfactory for field inoculations. In studies of the effect of pH on wilt infection, it was found that higher infection was obtained at pH 5.5 to 5.7 than in the alkaline ranges or in those that tended toward alkalinity in Ruston sandy loam. In Lintonia silt loam the most favorable soil moisture for infection was 60 per cent of the water-holding capacity.

Air-dried Oat-wheat Mixture for Contaminating Soil with the Cotton-wilt Organism, Fusarium vasinfectum. A. L. SMITH. Comparative studies of freshly prepared and air-dried oat-wheat mixture for contaminating field soils with *Fusarium vasinfectum* indicates an equal or slightly superior infective power of the air-dried mixture. The air-dried material was produced and dried during the winter months and stored until spring. Both materials were produced in 24-gauge, galvanized-iron containers of 1 bu. capacity equipped with 4″ screw top with ½″ opening for plugging, a ¼″ opening for pressure equalization during sterilization, and handles.

The mixture, consisting of 3 parts oats and 1 part wheat, was placed in the containers hydrated by soaking 12 hours in an excess of water, drained, sterilized and seeded with 250 cc. of spore suspension. After 10 days' incubation the mixture was emptied in bushel baskets and incubated an additional 48 hours before placing in seed-drying trays. After drying to 14 per cent moisture, the material was resacked and stored until used. Early field applications placed in deep furrows (4″–8″ below the seed level) and well mixed with the soil gave best results. When applied at the rate of 685 pounds per acre, a maximum of 75 per cent of dead or wilted plants was obtained with Half and Half variety on soil previously having a very light wilt infestation.

A Promising Wilt-resistant Long, Staple Cotton. D. C. NEAL and C. B. HADDON. A selection of Delfos cotton (Delfos 2323–965–425) made at the Northeast Louisiana Experiment Station in 1934 has exhibited marked resistance to Fusarium wilt in tests conducted for the past 3 years on heavily infested soil at Baton Rouge, Louisiana.

At Baton Rouge this selection produced over 1100 pounds of seed cotton per acre, and in a comparative test with 6 standard varieties at St. Joseph, La., in 1937, it ranked second in production, yielding 2246 pounds of seed cotton per acre. On bluff soil the staple averages 1⅛ inches and from 1 5/32 to 1 3/16 inches in the Delta, with about 32.5 per cent of lint.

Fertilizers in Relation to Incidence of Wilt as Affecting a Resistant and a Susceptible Variety. JAMES B. DICK and H. B. TISDALE. In 1937 the Division of Cotton and Other Fiber Crops, cooperating with the Alabama Experiment Station, began a study to determine the effect of different combinations of nitrogen, phosphate, and potash upon the incidence of cotton wilt. On the basis of 600 lb. per acre, 3 levels of nitrogen (0, 6, 12), phosphorus (0, 8, 16), and potash (0, 4, 8) were used in a factorial design, thus giving 27 fertilizer combinations. Two varieties, Cook 307 (resistant) and Half and Half (susceptible) were used. The experiment was planned to continue for 3 years. The first season's results indicated that:

1. While there is a high inverse correlation between yield and the number of plants affected by wilt, some fertilizer combinations have an additional effect on yield.

2. Applications of nitrogen and potash alone, and especially in certain combinations, effectively reduce wilt and increase yields. There is a differential response of varieties to fertilizers under conditions of severe wilt. The use of phosphate materially increases

the incidence of wilt, particularly so in the absence of adequate amounts of nitrogen and potash. Even when the latter are present it appears that increased wilt largely nullifies the beneficial effects of phosphate on yield.

Cotton Varieties in Relation to Cotton Wilt. H. B. TISDALE and JAMES B. DICK. The Alabama Experiment Station, cooperating with the Division of Cotton and Other Fiber Crops and Diseases, began studies in 1936 on the relation of cotton varieties to the cotton-wilt diseases. The results of these studies for 1926 and 1937 indicate:

1. No evidence for the existence of different physiological strains of the wilt organism in Alabama despite marked differences in virulence for location and conditions. Classification of varieties into resistant, highly tolerant, weakly tolerant, and susceptible groups, based on relative resistance appears to be unaffected by severity, although in one test the resistant group may have as much wilt as the weakly tolerant group at another location.

2. The varieties of cotton tested differ in their response to varying applications of potash. Resistant varieties are benefited more by moderate applications of potash than are the susceptible varieties. The latter apparently require more potash and are indifferent to excess applications, while resistant varieties require less potash and show injury and delayed maturity with excess applications. A gradation of these requirements from the most susceptible to the most wilt-resistant varieties suggests relationship between wilt resistance and ability to use potash.

Field Studies on Fusarium Wilt of Cotton in Arkansas. The relation of "wilt" and "total infection" as influenced by potash fertilization. E. M. CRALLEY and W. H. THARP. Three varieties of cotton—resistant, tolerant and susceptible—were tested in a randomized arrangement at Ozark in 1936 and LaGrange in 1937. At Ozark, potash was applied in the seed bed 10 days prior to planting, and as side dressing 1 month and 2 months after planting. But 1 application of potash was made at LaGrange, it being placed in the seed bed 21 days prior to planting. Wilt and infection data were obtained by pulling and examining plants at different periods during development.

The data were recorded in terms of both wilt and infection, and a wilt-index method has been employed to facilitate measurement of relative disease severity.

The results from the 2 years' work indicate that under the conditions of these experiments: (1) infection occurs progressively with advance in season in resistant, tolerant, and susceptible varieties; (2) significant differences in relative disease severity between potash-fertilized and nonfertilized plants are accentuated only late in the growing season; (3) applications of potash after planting were decreasingly effective with lapse of time between planting and application.

The Effect of Heavy Metals and Minor Elements upon the Growth of Phymatotrichum omnivorum in a Nutrient Solution. L. M. BLANK. A study has been made of the effect of some heavy metals and minor elements upon the growth of *Phymatotrichum omnivorum*. Included were copper, iron, manganese, zinc, aluminum, boron, silicon, cobalt, fluorine, iodine, lithium molybdenum, nickel and mercury. To a nutrient solution purified by treatment with calcium carbonate, the metals or minor elements were added at various rates. The dry weight of the fungus mat after 28 days incubation at 28° C. has been used as the criterion of the effect of the various elements.

The factorial design was employed. The data are being treated statistically. From the analysis so far made, high significance is evident for the stimulating or inhibiting effects of certain metals at given concentrations. The design permits evaluation of their effects independently and in various combinations. Slides showing the analyses of two experiments illustrate significant main effects and interactions resulting from combinations of 2 or more metals. Since the design may be of interest to other investigators, its efficiency and the method of analysis is shown. Until more of the data are analyzed, conclusions will not be attempted for the possible rôle of these elements nor for their necessity or concentration for optimum growth requirements.

Some Studies on Phymatotrichum Root Rot. WALTER N. EZEKIEL, J. J. TAUBENHAUS, and J. F. FUDGE. Monocotyledonous plants apparently owe their general immunity from root rot to an acidic, ether-soluble material in the roots. Fractions containing this have been prepared from onion, gladiolus, cane, canna, Hemerocallis, and Johnson grass. A possible perfect stage appeared in transfers of *P. omnivorum*, previously carried for 6½ years in culture. In drop cultures, hyphae of this lamellate or poroid basiodomycete anastomosed with those of known *P. omnivorum*. Laboratory experiments showed that pentachlorethane, tetrachlorethane, and xylene were highly toxic and readily penetrated moist soil. However, these volatile fungicides have not eradicated root rot from infested plots and are not, as yet, recommended for practical use against Phymatotrichum root rot.

Soils originally acid or acidified by various chemicals are less favorable for infection or survival of root rot than neutral or alkaline soils. In acidifying soils to attempt control of root rot, the reaction of the surface soil layer appeared particularly significant. Barriers of sorghum plants prevented spread of root rot in cotton fields, and are recommended to block invasion of uninfested areas and to border experimental plots.

Cotton Root Rot in Texas in 1937, and Conditions Affecting Its Local Prevalence. WALTER N. EZEKIEL. Estimates of losses to the 1937 cotton crop from Phymatotrichum root rot were made on September 20–24 for 770 fields, in 22 counties. Mean percentages of plants killed in different counties varied from 0 to 29.1 per cent. For different portions of the State, reduction in yield was estimated at 0 to 12.1 per cent, and for the State, about 5.9 per cent or over 300,000 bales. In 10 selected counties with soils approximately equally favorable for the disease, root-rot losses correlated highly with April, May, June, and July rainfall and normal mean annual temperature. Increase of 1 inch of rainfall in April or May was associated with increase of about 14 per cent of root rot; in June with about 20 per cent; and in July with about 32 per cent. Differences in August rainfall were apparently without effect. Temperature increase of about 1° F. was associated with an increase of about 1.1 per cent of root rot. The disease is not prevalent in sections of Texas with mean normal temperatures below about 60° F.

REPORT OF THE TWENTY-SECOND ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The twenty-second annual meeting of the Pacific Division of the American Phytopathological Society was held in Balboa Park, San Diego, California, June 21–23, 1938, in conjunction with the meetings of the Pacific Division of the American Association for the Advancement of Science.

The three half-day sessions for the presentation of papers were attended by from 30 to 50 pathologists from California, Oregon, Washington, Arizona, and Hawaii. Twenty-six papers were presented, the titles and abstracts of which follow this report.

Wednesday afternoon was devoted to excursions in the vicinity of San Diego, the largest group visiting the Scripps Institution of Oceanography at La Jolla. A short informal symposium on the teaching of plant pathology was held Thursday afternoon. The same topic has been suggested for a more extensive program at the next annual meeting.

At a business meeting the following officers were elected:

W. T. HORNE, President, University of California Citrus Experiment Station, Riverside, California.

B. F. DANA, Vice President, U. S. Department of Agriculture, Corvallis, Oregon.

L. D. LEACH, Secretary-Treasurer, University of California, Davis, Calif.

EUBANKS CARSNER, Councilor, U. S. Department of Agriculture, Riverside, California.

M. W. GARDNER and L. D. LEACH, Members of Council of Pacific Division of A.A.A.S.

The next annual meeting will be held at Stanford University, Palo Alto, California, during the third week of June, 1939.

Movement of the Virus of Sugar Beet Mosaic. C. W. BENNETT. The virus of beet mosaic moved inward from the distal end of beet leaves a distance of 12 inches in 72 hours. No virus was obtained from opposite noninoculated leaves of these plants over a period of 4 weeks following inoculation. Invasion of large crowns from inoculated small shoots of the same plants was relatively slow. Movement in the reverse direction was much more rapid. When infection was produced in one shoot of a plant having 3 crowns separated by distances of 12 inches or more, the virus required a relatively long time to invade either of the other 3 shoots when they were growing normally. However, when 1 of the noninoculated shoots was defoliated or placed in the dark for 5 days the virus invaded it and produced symptoms within a short time. Using plants with 3 shoots, one was inoculated with the virus of curly top and another with the virus of mosaic, the third shoot being held as a check on relative rate of movement of each of the 2 viruses into it. Mosaic symptoms appeared on the inoculated and check shoots in average times of 8 and 51 days, respectively. Symptoms of curly top appeared in average times of 15 and 181 days, respectively. Other evidence indicates that when the 2 viruses are introduced simultaneously into a shoot of a plant bearing 2 or 3 shoots, the virus of mosaic invades the noninoculated shoots ahead of the curly-top virus. This is believed to be due to the ability of the virus of mosaic to move in both phloem and parenchyma, whereas movement of the virus of curly top probably is confined to the phloem.

Serological Differentiation of Citrus Red Scale, Aonidiella aurantii and Citrus Yellow Scale, A. citrina. F. R. BUSHNELL and L. J. KLOTZ. By precipitin technique it was found possible to distinguish between *Aonidiella aurantii* and *A. citrina.* Eight grams of the living *A. aurantii* were ground in a mortar with quartz sand and extracted by shaking in a 100 cc. solution of 10 per cent NaCl. The extract was dialyzed to remove the salt, and the resulting precipitate was dissolved in 0.85 per cent NaCl solution. This material was injected intravenously and intraperitoneally into rabbits at 3-day intervals and the antiserum recovered 8 days after the last (8th) injection. Antigen of the red scale (*A. aurantii*) in a dilution as high as 1: 13 gave an abundant precipitate with this antiserum. Antigen of living yellow scale (*A. citrina*) gave no precipitate at any dilution used. One lot, labelled yellow scale, did give a reaction in a dilution as high as 1: 6, but later proved to be a mixture of red and yellow scale. While recent studies show that the 2 species may now be readily distinguished by microscopic morphology, thus superseding the possible usefulness of serological technique in taxonomy of these insects, the discovery that such closely related insects are seriologically different suggests a usefulness of the technique in this field. Extracts of dead Argentine red scale (*C. aurantii*) from tung oil trees and dead Asiatic red scale (*C. aurantii*) from roses gave no precipitin reaction with citrus red scale antiserum at any dilution. This suggests that if these 3 lots of scale are the same species, food or death (or both) of the insects alters their proteins.

The Present Status of Curly-Top Resistance in Sugar Beets. EUBANKS CARSNER. The curly-top-resistant sugar beet, variety U. S. 12, combines very high resistance with satisfactory sugar content. In a test under severe curly-top exposure its calculated acre yield of indicated-available *sugar* was 1.7 tons as compared with a total acre yield of *roots* of 1.6 tons from the European variety, R. & G. Old Type. Under less severe exposure, where R. & G. Old Type yielded 10 tons of beets per acre, U. S. 12 gave 23.7 tons of beets and 3.2 tons indicated-available sugar per acre. U. S. 12 bolts readily with fall planting in California, but, where spring planting is practiced, it shows little or no bolting.

U. S. 33 is characterized by moderate curly-top resistance and high sugar percentage. The bolting tendency is high.

U. S. 14 is low in bolting tendency and moderately resistant to curly top. Adaptability of the variety is restricted because it is very susceptible to downy mildew.

The 3 varieties mentioned will continue in commercial use for at least the next 2 years and the extent of their use will be in the order they are listed here.

Further Studies of the Host Relationship of Peach Mosaic in Southern California. L. C. COCHRAN and LEE M. HUTCHINS.

A Study of the Pathological Anatomy of Psyllid Yellows with Special Reference to Similar Changes in Sugar Beets Affected with Curly Top. J. R. EYER and MAYBELLE MILLER. In a histological study of material from plants affected with psyllid yellows, 4 abnormalities were found. Phloem necrosis occurred in stems, stolons, roots, and lateral rootlets, being most severe in stems and stolons. Pseudocalluses in the sieve tubes, and an increase in number of sieve plugs also were observed. Nuclear changes were prominent in the companion cells, phloem parenchyma, pericycle, and cortex. These manifested themselves in the form of the flaky appearance of the nucleoplasm, beaded membrane and hypertrophy and contortion of the entire body. None of these abnormalities was observed in histological sections from healthy plants.

Artschwager has reported very nearly the same malformations in his study of sugar beet seedlings affected with curly top.

Transmission of Psorosis of Citrus. H. S. FAWCETT. Since the discovery of leaf symptoms of psorosis in 1933, on the same trees with the previously known scaly bark symptoms, much new evidence has accumulated as to the apparently limited means of transmission of psorosis. Rubbing with crushed tissue or extracts of diseased plants, injected filtered extracts, etc. have failed to transmit this disease. No vector has yet been found. Investigations indicate that transmission from contiguous trees in nursery rows or in the orchard is unimportant in California. Transmission experimentally has been made only by the fusion of live cells of the diseased buds, bark, or root grafts with the live cells of healthy plants. Recognition of the leaf symptoms has made it possible to determine that other citrus species, not exhibiting the bark symptoms, may be carriers of the virus. Many of these carriers have considerably more tolerance to the presence of the virus than the varieties showing bark symptoms, such as sweet orange, grapefruit, and tangerine. The transmission of psorosis by budding to various species, varieties, and hybrids of citrus has been obtained in numerous tests. Knowledge of leaf symptoms, as well as bark symptoms, is being applied in commercial practice to avoid transmission in budding by the employment of virus-free sources for bud wood for the propagation of trees for new plantings.

Types and Symptoms of Psorosis and Psorosis-like Diseases of Citrus. H. S. FAWCETT and L. J. KLOTZ. Two types, psorosis *A* and psorosis B, have similar bark symptoms, the latter with more rapid spread and with gum formation, often much ahead of the bark eruptions. Leaf symptoms in the form of small, light areas about 1 to 3 mm. on young, rapidly growing leaves are similar in the *A* and *B* types but in older, mature leaves the *B* type may show large chlorotic ring spots approaching those of zonate chlorosis in Brazil, while the *A* type shows only occasional small circular or semicircular ring spots. Symptoms on fruit are rare in the *A* type but in the *B* type are often large, discolored circular to semicircular rings or grooves, the sunken tissue varying from pale yellow to brown, with occasional necrotic breakdown. In fruits of grapefruit many irregularities often may result in bumpy, misshapen fruit. Concave gum disease has concavities in the bark, with gum-infiltrated layers in the wood and flecking type of symptoms on young leaves, similar to those of psorosis *A* and *B*. It is possible that *A* and *B* are variations in the effect of the same virus and that the concave gum disease is closely related. The leprosis in Florida was bark symptoms on older twigs, somewhat similar to psorosis, but no definite leaf symptoms in the rapidly growing leaves have been noted. The circular spots on mature leaves and on fruit are distinctly different from those of either psorosis *A* or *B*. The concentric ring spot of South Africa has spots on leaves and fruit, with certain resemblances to some of the spots occurring in leprosis. It is suspected that several of these diseases are due to the same virus or closely related viruses.

Effect of Sodium Citrate on Release of Curly-top Virus from Alcoholic Precipitate of Plant Juice. J. M. FIFE. Sodium citrate was very effective in releasing the curly-top virus from the alcoholic precipitate of sugar-beet leaf juice or tomato-plant juice. Maximum infection was obtained with 6.8 mM. sodium citrate. Where the extractable virus content of the plant juice is low, as shown by the percentage infection obtained with water as the extracting solution, the sodium citrate method of extracting the virus is far superior to the method in which water alone is used. The virus is not inactivated in the juice at pH values as low as 2.2 because infection was obtained with sodium citrate but not with water alone.

Studies of Selected Strains of Curly-top Virus. N. J. GIDDINGS. Four curly-top-virus strains designated as 1, 2, 3, and 4 have been recognized by the differential reactions of varieties of sugar beet, *Beta vulgaris*. Percentages of plants infected and severity of symptoms were used as bases of comparison. Virus strains 1 and 3 induced severe symptoms in susceptible beets; but strain 3 seldom infected the resistant beet used, while strain 1 infected a high percentage of resistant beets and induced very obvious symptoms. Strains 2 and 4 induced mild symptoms in susceptible beets; strain 4 infected only a small percentage of the resistant beets, while strain 2 infected a high percentage of them but induced very mild symptoms. The highly resistant beet variety, 1167, showed distinctive reactions to each of the 4 virus strains, and the differences were highly significant statistically. Tobacco, *Nicotiana tabacum*, and tomato, *Lycopersicon esculentum*, were not infected by virus strains 2 or 4. Plantain, *Plantago erecta*, peppergrass, *Lepidium nitidum*, and Great Northern variety of bean, *Phascolus vulgaris* L., were not infected by virus strain 2. Susceptible beets infected with the less virulent strains were not immunized from the more virulent strains but appeared to be rendered more susceptible to severe injury by such prior infection.

A Plea for Boldness. J. LEE HEWITT.

Species of Sclerotinia Causing Brown Rot of Deciduous Fruits in California and Their Distribution. WM. B. HEWITT and L. D. LEACH. Brown rot of deciduous fruits is one of the most important disease-control problems of the California growers of stone fruits. It has been essentially one of blossom and twig blighting with an occasional year of fruit rotting, and has occurred mostly in the coastal regions in apricots, almonds, cherries, and prunes. In the past few years, however, large amounts of brown rotting of peach fruits have occurred in the peach-producing areas of the interior valleys.

On March 3, 1936, apothecia of *Sclerotinia fructicola* were found developing from mummied peach fruits in the peach orchards of the upper Sacramento Valley, apparently the first time they have been found or reported in the State.

Sclerotinia fructicola was compared with cultures isolated from blighted apricot twigs. The 2 cultures were different in gross culture characteristics, and the latter was identified as *S. laxa* Adhr. and Ruh.

A survey of the deciduous-fruit districts of California was made for fungi causing brown-rot blossom and twig blight and fruit rotting. In so far as the survey has been made, only 2 species, *Sclerotinia fructicola* and *S. laxa* have been found.

Inheritance in Cucumis melo of Resistance to Powdery Mildew (Erysiphe cichoracearum). I. C. JAGGER, T. W. WHITAKER and D. R. PORTER. Powdery Mildew Resistant Cantaloupe No. 45, now the leading variety in the Imperial Valley of California, was developed from a cross between the very susceptible variety Hales Best and a highly resistant plant selected from an unfixed variety from India. This resistance seems to be inherited as a simple Mendelian dominant factor. In the F_1 generation from crosses between homozygous resistant and homozygous susceptible plants, all plants show the same high degree of resistance as the resistant parent. In a series of records on the F_2 generation, the resistant plants totaled 233 and the susceptible 86. This approaches the expected 3-to-1 ratio. Back crosses between F_1 plants and the susceptible parent gave 21 resistant to 25 susceptible plants, which is within the range of expectation for a 1-to-1 ratio.

These data are based on records made in Imperial Valley during the early part of the season of 1938 and in preceding seasons. In midseason of 1938 a new biologic form of the mildew appeared in that section, to which the Resistant No. 45 variety shows, at least, considerably susceptibility. No data are available on resistance to this new form.

The Nature of Water Damage to Citrus Fruits. L. J. KLOTZ. Water injury to citrus fruits is important mainly on navel oranges, which mature during the rainy season. Experimentally, however, any of the common species and varieties of citrus can be damaged. The first evidence of injury is the appearance of minute cracks or checks in the cuticle and one or more layers of underlying cells in the vicinity of the navel convolution, wounds or shoulder. Fresh wounds in the rind such as those caused by thorn and twig punctures and abrasions, sand, hail, and wind or injury by such agencies as frost and oil sprays are important in increasing the incidence of water spot. Old calloused or healed rind scars, as those initiated by thrips, tortrix, katydids, rubs, scratches, and chemical burns during the period of rapid growth of the young fruit do not affect the incidence of water spot. The cracking or checking stage is followed by a rapid entrance of external water evidenced by a water soaked, blistered-like area, which is readily invaded by blue and green molds and decayed. The water taken up by the hydrophilic albedo cells affects the permeability of the cells surrounding the oil glands and the toxic oil is liberated into the albedo. If dry weather follows the initial stages of the trouble the tissues dry down leaving an ugly brown, collapsed area that becomes covered by a dark *Cladosporium* sp. when wet weather recurs. The more mature the fruit the more readily it is damaged, for the rind becomes less elastic and, because of increase in sugars, has a greater osmotic value. Kumquats, which are very readily and severely damaged by rains, have a greater osmotic value and pectin content in the sap of the rind than do navels and Valencias.

Curly-top Virus in Root Tips of Sugar Beets and Beans. C. F. LACKEY. Curly top has been found to be translocated through the phloems of its host plants. Studies made on root tips of sugar beets and beans show that the virus also is found below the protophloem sieve tubes in the meristematic region. A method has been developed by which sections .2 to .5 mm. can be cut from fresh root tips and tested for the presence of virus. These tests show that a much higher percentage of infection on susceptible test beets can be obtained from tissue below the protophloem sieve tubes than from sections including them or from checks consisting of entire rootlets 5 mm. long. When four sections were cut from tips, each .5 mm. long, twice as much infection was produced with virus from the third section than from the second, but less than from the root cap section. Similar results were obtained from diseased bean-root tips, although there was not so much difference in percentage of infection between the 3 lots of tissue. Degeneration of the pericycle of the root tips follows the sieve tubes closely. A very few cases of degeneration of cells in the meristematic region directly below the extreme end of the sieve tubes have been found. Usually there is no visible evidence of the virus in this region of the root tips.

Seed Treatment for the Control of Damping Off of Sugar Beets in Northern California. L. D. LEACH and B. R. HOUSTON. Isolations indicate that soil-borne *Pythium* sp. and *Rhizoctonia solani* are the most important organisms causing damping off of sugar beets in northern California. *Phoma betae* is frequently destructive on seedlings from European grown seed, but has not been observed on seedlings from domestically produced seed. This relation was confirmed by laboratory examination, which showed that certain lots of European seed carried considerable Phoma infection, whereas none was observed on domestic seed. Greenhouse and field trials indicate that cuprous oxide is an effective seed treatment where damping off is caused by *Pythium* sp., but that organic mercury compounds (Ceresan and New Improved Ceresan) are more effective when *Rhizoctonia* and *Phoma* are involved. In severely infested fields the uniformity, as well as the density of thinned stands, has been improved by seed treatment, as in the following example: non-

treated—beets per 100 feet, 67.6, coefficient of variation, 71.4 per cent; treated—beets per 100 feet, 96.7, coefficient of variation, 46.8 per cent.

Pythium Disease of Fibrous-rooted Begonia. J. T. MIDDLETON, C. M. TUCKER, and C. M. TOMPKINS. A disease affecting the crown, basal portion of the stem, and the leaves of fibrous-rooted Begonia plants, variety *Fire Sea*, was observed on bedding plants in Berkeley, California. A similar disease was found on fibrous-rooted Begonia plants, varieties *Carmine, Christmas Cheer,* and *Primadonna,* grown in greenhouses at Columbia, Missouri.

Symptoms of the diseases are identical for the varieties mentioned. In naturally infected plants the crown and lower portion of the stem become somewhat discolored, water-soaked, and soft. Infection may progress up the stem 2 or 3 inches, causing the stem to collapse. In infected leaves the petioles become limp and dark, the laminas water-soaked and flaccid; subsequent abscission of infected leaves is not uncommon. When potted plants were grown in close proximity with one another the disease was quite serious, being distributed throughout the plot by means of leaf infection.

Pythium ultimum was isolated from the Berkeley material. *Pythium de Baryanum, Pythium splendens,* and *Pythium ultimum* were isolated from the Columbia material.

All of the *Pythium* isolates proved pathogenic to the Begonia varieties mentioned by adding the fungus to the soil; each organism induced the disease individually.

Cucurbit Powdery Mildew on Carica Papaya. P. A. MILLER. A powdery mildew attacking seedlings of *Carica papaya* in the greenhouse at Los Angeles was first observed in November, 1935. Cross-inoculation experiments, using conidia from papaya plants on dwarf sunflower plants and conidia from mildewed sunflower plants on healthy papaya seedlings, resulted in no infection. In the summer of 1936, papaya seedlings grown in the greenhouse at Riverside were successfully inoculated using conidia from field-grown cucumber plants. All of the plants inoculated developed sporulating mildew spots on the cotyledons, stems, and leaves. Cucurbit plants in the greenhouse at Los Angeles were inoculated with conidia from these mildewed papaya seedlings. The mildew that developed on the inoculated cucurbit plants spread to the lower leaves of large mature papaya plants growing in the same greenhouse. In October, 1936, the lower leaves of mature papaya plants growing in the open in Orange County, California, were found attacked by cucurbit powdery mildew. Mature leaves and plants are only slightly affected, but young seedlings may be severely injured by the disease. The results of the cross inoculation experiments and field observations indicate that *Carica papaya* is a host of the physiologic race of cucurbit powdery mildew common in this region.

Diseases of Ornamental Plants in Southern California. P. A. MILLER. A pythiacious type of fungus was found to be the cause of a gummosis affecting young *Acacia floribunda* trees in the Santa Monica region. Although the organism has not been definitely identified, inoculations with it have produced typical gummosis lesions. All stages of the root-knot nematode, *Heterodera marioni*, have been recovered from hypertrophied roots of *Tritoma* sp. and *Passiflora mollissima.* Brazilian Pepper, *Schinus terebinthefolius,* trees affected with *Verticillium* hadromycosis were found near Arcadia, California. An extensive planting of *Centaurea, Calendula* and *Ranunculus* plants on the same property were found severely attacked by *Sclerotinia sclerotiorum.* An oil-burning race-track drier, which covered an area 8×12 ft., was used to burn over 8 acres of level planting. Four minutes' exposure raised the temperature of the soil 1 in. below the surface to approximately 600° F. Sclerotia on the soil surface were reduced to ashes and those 1 inch below the surface did not germinate after a 4-minute exposure to heat from the burner. The soil of other areas was treated with 1–100 formaldehyde solution at the rate of 1 gal. per square foot after the plants had been removed. About 50 per cent of the sclerotia tested after exposure to this treatment did not germinate.

A Possible Significance of the Blue Nuclei of the Black Tulip. FRANK P. MCWHORTER.

Susceptibility of Cupressus and Allied Species to Crown Gall. CLAYTON O. SMITH. Artificial inoculations with cultures of the crown-gall organism, *Bacterium (Phytomonas) tumefaciens* on different species of the Cupressaceae showed that *Libroceđrus decurrens, Thuja orientalis, T. plicata, T. occidentalis* and *Thujopsis dolabrata* produced galls 5–25 mm. in diameter. The following species of *Cupressus* were received as small seedlings (except *C. lusitanica* and *C. sempervirens*) from Rancho Santa Ana Botanic Garden, California: *C. arizonica, C. bakeri, C. duttoni, C. forbesi, C. goveniana, C. macrocarpa, C. macnabiana, C. pygmaea, C. sargenti, C. thurifera, C. lusitanica* SPI 73844, and *C. sempervirens.* Galls 15–25 mm. in diameter developed on the above species of *Cupressus.* The galls on *Cupressus* are at first globose and have a normal bark, but with age (about 1

year), the galls may become rough and then resemble crown gall as it develops on other susceptible hosts. *Juniperus virginiana, J. procera* SPI 60553 are susceptible, while *J. cedrus* SPI 57080 and *J. californica* did not develop galls when inoculated. Inoculations on *Chamaecyparis lawsoniana* gave overgrowths that are not entirely typical of crown gall.

Pink Disease, a Bacteriosis of Pineapple Fruits. C. H. SPIEGELBERG. A pink to brown internal discoloration of ripe pineapples, often with cantaloupe odor, is a bacteriosis, as shown by isolations and inoculations. Faint discoloration beginning near the normal-appearing shell, extends laterally and inwardly, darkening and softening tardily. Heating browns affected tissues and juice; $(NH_4)_2HPO_4$ accelerates and intensifies the discoloration.

Pink disease occurs chiefly from January to March in low-acid fruits, but highly acid fruits are susceptible when inoculated, both in summer and winter.

The pathogen, variable in colony type, pigmentation, aroma, and pathogenicity, has also been isolated from field-collected surface-sterilized: *Calophyllum inophyllum, Citrus aurantifolia, Coffea* sp., *Eugenia malaccensis, Ficus macrophylla, Garcinia xanthochymus, Mangifera indica, Persea americana, Psidium cattleianum, P. guajava,* and *Terminalia catappa* fruits; also adult *Drosophila melanogaster, Carpophilus* spp. and pupal *Ceratitis capitata.* Fed to aseptically reared *Drosophila* larvae, it was reisolated from larvae, pupae, and adults, but these adults failed to infect pineapple. Adult *Ceratitis* females, but not males, infected Anjou pear and tomato, indicating ovipositor inoculation. Results with pineapple, not a host of *Ceratitis,* are inconclusive. The pathogen, associated with *Ceratitis* ovipunctures, is more abundantly distributed in the above 11 fruits than in pineapple, indicating pineapple infections probably follow chance visitation of vector.

A Nursery Blight of Citrus Caused by Phytophthora citrophthora. R. B. STREETS. An investigation was made of the nature of an unusual disease of citrus nursery trees in which the leaves suddenly wilted and died and the stem became blackened in a zone 6 to 12 inches long, just above the bud union. The wood of the sweet seedling-orange scion was definitely discolored, and agar cultures of the wood and inner bark yielded a white, cottony fungus growth. The sour orange rootstock was not affected. The fungus proved indistinguishable in cultural characters and pathogenicity from *Phytophthora citrophthora.* This disease, therefore, was considered an apparently undescribed manifestation of brown-rot gummosis. The nursery trees were being held in a closely covered "lath house," and a rather heavy, wet soil had contributed to high humidity, producing conditions favorable for attack by this fungus. Several hundred trees were rendered worthless, but the non-infected trees were saved by the prompt application of Bordeaux mixture, special care being taken to spray the trunks well.

A Root Rot Disease of Citrus. R. B. STREETS. The sudden wilting and death of a number of trees in a 20-year-old grove of tangerines was investigated by J. G. Brown and the writer. It was found that, while the larger roots and branch roots of trees in intermediate and early stages of the disease appeared healthy, the smaller roots and feeding roots were dead. Cultures of the infected roots yielded a high percentage of colonies of *Fusarium,* of 2, or possibly 3, species, resembling those often found associated with dry root rot of citrus. The pathogenicity of these cultures is being tested and control measures that have yielded favorable results in case of Phymatotrichum root rot are being tried. Cultures made from the feeding roots of grapefruit, navel orange, and Valencia orange from trees showing symptoms resembling early stages of the tangerine disease and resembling our citrus "decline" yielded the same species of *Fusarium.*

Control Measures for Phymatotrichum Root Rot of the Pecan. R. B. STREETS and LLOYD BRINKERHOFF. In the treatment of visibly infected pecan trees, ammonium sulphate alone, apparently satisfactory in many cases, sometimes failed to permanently check root rot under the most severe conditions. Ammonium phosphate (16–20) appeared definitely more promising, and will be tested extensively during the coming season. Sulphuric acid solutions showed definite promise when injected into the soil to a depth of 4 feet, either alone or in combination with ammonium sulphate or ammonium phosphate. The immediate effect is of much value in treating visibly infected trees. Sulphur alone, or in combination, injected or mixed with the surface soil, gives similar but much slower response because of the 3 to 6 weeks necessary for oxidation. Its value, therefore, is preventive rather than curative.

Tree-by-tree mapping of 5 large infected groves totalling 590 acres, and remapping after 5 and 12 months, respectively, demonstrated clearly that it is practical to detect trees slightly infected with root rot long before the symptoms become apparent to the untrained observer. This has made possible the treatment of trees in the first rather than the last stages of the disease with correspondingly greater success.

Effects of Soil Treatments for the Control of Phymatotrichum Root Rot on the Soil and the Pecan. R. B. STREETS and LLOYD BRINKERHOFF. Over 600 soil samples from treated and nontreated soils were analyzed for total soluble salts, total nitrogen, nitrate nitrogen, phosphates, sulphates, and pH. Increases in nitrate nitrogen, total nitrogen, phosphates, and sulphates are much greater in the surface foot and second foot and progressively less in the third and fourth feet.

Applications of ammonium phosphate reduced the pH more than equivalent amounts of ammonium sulphate, but the principle toxic effect lies in the ammonium ion. Sulphur and sulphuric acid treatments probably depend upon the reduction of the pH, although the response of trees has exceeded that expected from changes in pH.

The pecan, at least in the soils tested, shows very great tolerance to the chemicals used for root-rot control, and the danger of injury by the usual dosages appears to be very slight. No injury was produced by less than 12 times the standard application (1 lb. to 10 sq. ft.) of ammonium sulphate applied as a single treatment, by less than 10 times the standard application of ammonium phosphate 16–20, or less than 3 gal. per sq. ft. of a 7 per cent (by volume) sulphuric acid solution.

Acquired Tolerance of Curly Top in Nicotiana Tabaccum. J. M. WALLACE. *Nicotiana tabaccum* plants commonly recover from curly top. Plants showing severe symptoms on terminal leaves frequently produce basal or lateral shoots entirely free of symptoms or showing only inconspicuous symptoms. In other instances recovery comes about in the production of less severely diseased leaves and tissues in the terminal shoots that have shown severe symptoms and retarded growth for a period of time. Cuttings grown from recovered plants have an acquired tolerance to curly top as evidenced by their resistance to reinoculation. Cuttings from healthy, previously noninoculated plants are easily infected and develop severe symptoms. Tolerance developed following infection with one strain of curly-top virus protected against inoculation with a second strain of virus. Whether or not the second strain became established in the plants has not yet been determined.